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A "UNITARIAN" VIEW OF VACCINIA HEMAGGLUTININ*

B. A. BRIODY

Since Nagler's discovery[11] of the hemagglutinin obtained from suspensions of vaccinial lesions on the chorio-allantois, a number of investigators have attempted to characterize it more precisely. Nagler[11, 12] and others[1-7, 13] have shown that antibodies which inhibit vaccinia hemagglutinin (V.H.) develop in man, rabbits, mice, and guinea pigs following infection with vaccinia virus, but it is not known if the V.H. itself is antigenic. Burnet and Stone[4] observed that V.H. was a substance distinct from the virus particle. Since V.H. was inactivated by *Clostridium welchii* α-toxin (lecithinase) in the presence of calcium ions, and since citrate, specific antitoxic serum, and lecithin inhibited this inactivation, Stone[14] concluded that V.H. contains a phospholipid, most probably lecithin, and that the enzymic breakdown of this part of the complex destroys its hemagglutinating properties. On the basis of an analysis of some physico-chemical and immunological properties of V.H., Chu[5, 6] concluded that it is distinct from, and apparently unassociated with, the elementary body, that it is not the L-S antigen, but that it represents a new and distinct antigen occurring in a relatively large particulate state. Since protamine and histones were found to inhibit vaccinia hemagglutination, Fisher[8] suggested that the red cell receptors for the phospholipid might be protein in nature.

From all of these observations, it would appear that many of the important properties of V.H. have been established. However, as Gillen, Burr, and Nagler[9] have pointed out, some of the reported findings[4-8] indicate the possibility that two hemagglutinins exist. These may be itemized as follows: (a) although most of the V.H. is found in the supernatant after high speed centrifugation, a trace of V.H. is consistently present in the sediment; (b) crude vaccinia virus suspensions presumably contain a heat labile and a heat stable component. Gillen, Burr, and Nagler,[9] in experiments designed to investigate the existence of two hemagglutinins, concluded that there are two hemagglutinating fractions in suspensions of chorio-allantoic membranes infected with vaccinia virus: (a) a soluble V.H. which is heat labile, not retained by Seitz filters, and not sedimented by centrifugation at 17,000 rpm for three hours, and (b) a V.H. which is more closely associated with the elementary body and which is heat stable, sedimented by centrifugation at 17,000 rpm, and retained by Seitz filters. In addition, Gillen, Burr, and Nagler[9] found that the two hemagglutinins differed in the type of agglutination which they produced, in the extent to

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which they were adsorbed by fowl red cells, and in the hemagglutinating titers which they showed at 37 C. and at 5 C.

Although our observations are in part confirmatory of the results of others, it appeared desirable to record certain experimental data which have led us to believe that V.H., as it occurs in preparations of chorioallantoic membranes infected with the Levaditi, the Noguchi, the CL, or the Nelson strain of vaccinia virus, is polydisperse—that is, it has a range of weights. This "unitarian" view of V.H. is based on a number of considerations. In agreement with Chu,[8] we have observed that purified suspensions of elementary bodies, prepared from infected membranes by differential centrifugation combined with repeated washing and resuspension of virus, fail to agglutinate susceptible fowl red cells. The V.H. activity found in the sediment obtained in the first angle centrifugation at 5,000 rpm for one hour may be due either to the adsorption of V.H. onto the virus, or more probably to the presence of aggregates of V.H. which are similar in size to the virus. At any rate, it is abundantly clear that the titer of V.H. is not correlated with the infectivity of a suspension of elementary bodies (E.B.). For example, a crude suspension of E.B. prepared by sedimentation at 5,000 rpm for one hour, and a preparation of E.B. which had been washed repeatedly and resuspended, were adjusted so that each contained a 50 per cent infectious dose ($I.D._{50}$) of 10^9 per ml. for chick embryos. The crude suspension had a V.H. titer of 32 and the purified material a titer of less than one. The supernatant resulting after three successive centrifugation cycles at 5,000 rpm for 30 minutes (a procedure which removes the bulk of the virus) was subjected to high speed centrifugation in a refrigerated angle head centrifuge at 13,000 rpm for three periods of one hour each. The V.H. in the supernatants remained at the original titer of 64. The high speed sediments, when made up to original volume, had $I.D._{50}$'s of less than 10^5, and V.H. titers of 16, 2.5, and less than one after the first, second, and third periods respectively. These data suggest that there is no striking homogeneity of particle size of V.H., and that an extremely small quantity of active material is required to produce visible hemagglutination. This latter impression is borne out by the fact that chicken cells in one per cent suspension, when added to an equal volume of V.H., absorb between 10 and 15 units, while under the same conditions they will absorb approximately 600 units of influenza hemagglutinin. One unit of hemagglutinin is defined as the amount contained in the highest dilution which produces visible agglutination of chicken erythrocytes.

After the bulk of the virus is removed from suspensions of infected membranes by three 30-minute periods of centrifugation at 5,000 rpm, the remaining supernate is spun at 13,000 rpm for two hours. This last centrifugation separates V.H. into two fractions with strikingly different properties. The differences summarized in Table 1 were ascertained after the sediment and the supernatant had been adjusted to contain the same number of

hemagglutinating units. The heat stability of V.H. in the sediment agrees with that obtained by Chu[5] and by Gillen, Burr, and Nagler,[9] while the heat lability of V.H. in the supernatant confirms the observation of the latter workers. Trypsin itself, in the concentrations employed, agglutinated chicken erythrocytes, but its titer was only one-tenth that of the V.H. employed. The hemagglutinin remaining after the action of trypsin on V.H., was reduced in activity to one-half by normal rabbit serum and completely

TABLE 1

PROPERTIES OF VACCINIA HEMAGGLUTININ (V.H.) AS IT OCCURS IN THE SEDIMENT AND THE SUPERNATANT AFTER CENTRIFUGATION AT 13,000 RPM FOR TWO HOURS*

Property	*V.H. in sediment*	*V.H. in supernatant*
Effect of heat	Heat stable†	Heat labile‡
Effect of trypsin	Increases agglutinating titer five-fold	Increases agglutinating titer less than two-fold
Extraction with alcohol-ether	Increases agglutinating titer ten- to twenty-fold	Increases agglutinating titer less than three-fold
Lipid phosphorus	Approximately 3 times as much as in supernatant	Approximately one-third as much as in sediment
Effect of 0.1% $CaCl_2$ and 0.5% $MgCl_2$	Inhibits 4 to 8 units of V.H.	Inhibits 4 to 8 units of V.H.

* The bulk of the virus had previously been removed. See text for details.
† There was no marked reduction when heated to 70° C. for 30 minutes.
‡ Completely inactivated when heated to 56° C. for 30 minutes.

inhibited by specific anti-vaccinia rabbit serum. This suggests that trypsin may have partially inactivated the virus antigen, thus causing the release of phospholipid units which are capable of agglutinating chicken erythrocytes, but which are inhibited by normal serum. The hemagglutinin obtained by the extraction of the V.H. with alcohol-ether was entirely "non-specific" in that it was completely inhibited by normal rabbit serum. The point of greatest interest in the treatment of V.H. with trypsin and in the extraction of V.H. with alcohol-ether is that the increase in hemagglutinating titer which resulted was much greater in the sedimented V.H. than in the V.H. of the supernatant. In addition, the amount of lipid phosphorus in the sedimented V.H. was approximately three times that found in the V.H. in the supernatant. Although these data do not establish the view that V.H. is polydisperse, they certainly suggest that this may be the case. The in-

hibition of V.H. by calcium chloride and magnesium chloride was observed to be the same for the V.H. in the sediment and that in the supernatant, suggesting a common mechanism of action. Calcium and magnesium ions have been found by Hilleman, Haig, and Helmold[10] to inhibit the non-infective hemagglutinin of the psittacosis-lymphogranuloma venereum group of viruses.

If V.H. is polydisperse, it would not be surprising to find differences in filterability, and in fact these have been observed by Chu[5] and Gillen, Burr, and Nagler.[9] There is no obvious explanation for the heat stability of V.H. in the sediment and its heat lability in the supernatant. These differences may be related to the phospholipid content of the V.H., which might function as a protective coating or which, more probably, might alter the spatial configuration of the V.H. complex. Thus, inactivation of V.H. in the supernatant might require one type of alteration of the molecular configuration of the phospholipid, while inactivation of the sedimented V.H. might require a different type due to its greater concentration of phospholipid. On the basis of the foregoing considerations, it is suggested that V.H. is polydisperse much as a substance such as hyaluronic acid is polydisperse—that is, it is found in a range of weights.

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FAVISM IN CHILDHOOD*

A Case Report

DONALD E. PICKERING† AND SIDNEY HURWITZ

"Favism" defines an acute hemolytic episode and its sequelae following ingestion of the bean or inhalation of the pollen of *Vicia faba* (broad bean or fava bean) in an individual probably sensitized by previous contact of a similar nature. The features of this syndrome and its potentially widespread occurrence in this country,‡ together with an excellent account of its prevalence in Italy, were brought to light in the American literature by Luisada in his review published in 1941.[4]

It is surprising that a total of only six cases of favism in childhood have been reported in the American literature to date.[1, 2, 3, 6, 7] All six cases followed ingestion of the bean rather than inhalation of the pollen. This infrequency, however, may be due to the fact that it is customary in this country to market the bean far away from the areas in which it is grown. In Italy, where the morbidity rate is much greater than in the United States, approximately 40% of the cases observed followed inhalation of the pollen.[4] The relatively infrequent opportunity for sensitization by way of pollen inhalation or ingestion of the freshly grown bean could well account in part for the infrequency of favism in this country where they are grown commercially in large fields attended principally by machines. Pazzi Demurtas[6] has shown that the antigenicity of the pollen is significantly greater than that of the fresh bean. He has also shown that the antigenicity of the fresh bean is significantly reduced by drying or cooking. Once a person has become sensitized, a very small quantity of the bean is probably sufficient to produce a reaction.

Symptoms usually become manifest within 24 to 36 hours after the bean has been eaten, although they may appear within a few seconds after inhalation of the pollen.[4] Luisada has stated that the early symptomatology consists chiefly of malaise, headache, dizziness, nausea, and vomiting followed by chill, fever, and often lumbar pain.[4] More specific signs referable

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‡ Ingestion of fava beans and favism have been common to the Italian and to other Mediterranean peoples for centuries, and those peoples have brought the bean and their appetite for it to this land.

Currently, fava beans are grown commercially in Mexico, Mississippi, Louisiana, Florida, and California for distribution over the United States. They are also grown on a small scale in New Jersey and in surrounding areas. During the harvest period of late April, May, and June as many as three carloads of beans arrive daily in New York according to the *New York Daily Fruit Report* (Duane Street, New York, New York).

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to the acute hemolytic episode may be listed as jaundice, hemoglobinuria, marked pallor, and anemia; these may be the first to appear in cases following ingestion of the bean. Varying degrees of hemoglobinuria usually persist up to a week, depending upon both the duration and degree of hemolysis present—factors which also determine the variable amount of blood required to reinstate and maintain the patient's red cell volume. The reaction of the patient to acute anemia and to the products of his hemolyzed red cells may be extreme. Listlessness, anorexia, palpitation, and sometimes respiratory distress are commonly seen along with joint pains, fever, flank pain, albuminuria, and urinary casts. Occasionally a transient hepatosplenomegaly develops during the acute process. Residual renal damage has not been reported to date. Most features of favism as defined above have been present in the cases reported in American children to date.

*Case report (R.V., Case #5177)**

Present illness. The patient was a 28-month-old male child of Italian descent, admitted to the Hospital of St. Raphael on April 30, 1951 at 4:30 p.m. He had been in seemingly good health until approximately eight hours before admission when his mother first observed him to be jaundiced, somewhat lethargic, and irritable. These features persisted, and during the day he developed a fever of 102° F. He had voided three times during the eight-hour period before admission and on each occasion it was noted that the urine was "dark brown" in color. His usual morning stool had been normal.

Specific questioning of the parents at the time of entry revealed that the child had ingested fava beans in the home of his maternal grandmother two months previously and again 36 hours before the onset of jaundice.

Past history and family history. This child was born in New Haven, Connecticut, following an uncomplicated full-term pregnancy and normal delivery. Growth and development had been normal. He had received basic immunizations. There was no history of previous illnesses nor manifestations of an allergic diathesis. His parents were both 32 years of age and were first generation Italian-Americans. They were in good health. A four-year-old female sibling had a history of intermittent bouts of urticaria following ingestion of tomato or of milk. There was no other family history of allergy. No family member had been known to have jaundice or anemia at any time.

Physical examination. Temp., 101.4°; pulse, 128; resp., 32. The child was a well-developed, well-nourished male weighing 32 pounds. He appeared acutely ill. There was marked generalized icterus of his skin and sclerae. His mucous membranes were pale. No significant lymphadenopathy was present. Examination of the heart and lungs was normal; there was a soft, grade-one, precordial, systolic murmur. The liver and spleen were not palpable and the remainder of the physical examination was noncontributory.

Laboratory data. The patient had a hemolytic anemia and the pertinent data are grouped on Chart I. Other data, not shown on Chart I, included a negative Mazzini and a negative Coombs' test. Neither sickling nor spherocytosis was observed on proper red blood cell preparations. Red cell fragility was normal. Bleeding and clotting time and prothrombin time were normal. There were 250,000 platelets per cu.mm. of peripheral blood. The Rumple-Leeds test for capillary fragility was negative. Serum

*We are indebted to Dr. Morris Y. Krosnick who referred this patient to the hospital.

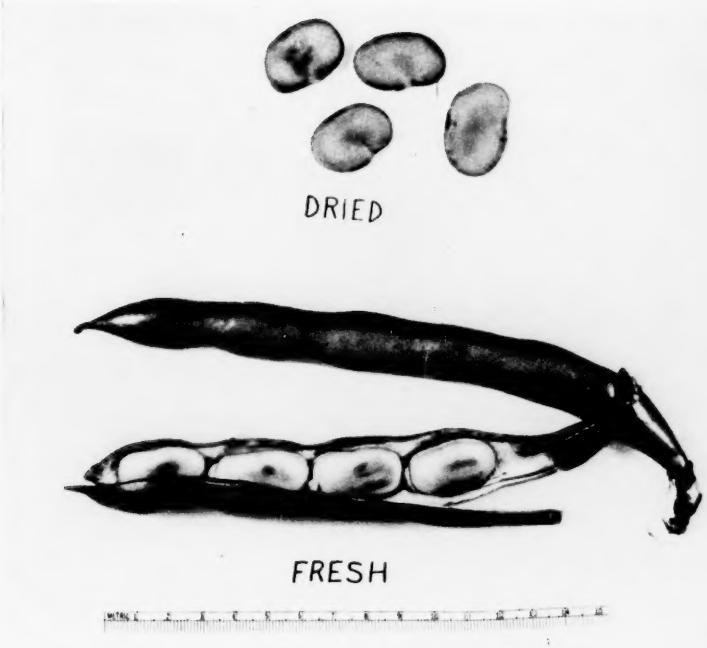

FIG. 1. Fava beans.

non-protein nitrogen was 27 mgm.%. Cephalin flocculation test and thymol turbidity were normal.

Inspection of bone marrow sampled from the iliac crest on the second hospital day revealed marked erythropoietic activity and an increase in eosinophils.

Course and discussion. The patient was transfused with three hundred cubic centimeters of citrated whole blood shortly after admission; there was no untoward reaction and the child concomitantly showed improvement.

CHART I

LABORATORY DATA DESCRIBING THE COURSE OF FAVISM IN A 28-MONTH-OLD ITALIAN CHILD IN WHOM JAUNDICE BEGAN 36 HOURS FOLLOWING INGESTION OF FAVA BEANS

		Time performed after the onset of jaundice (days)					
	Determination	*1/3*	*1*	*2*	*3*	*4*	*5*
Blood	Erythrocytes/cu.mm.	2.5 Mil.	3.1 Mil.	2.8 Mil.	2.9 Mil.	3.0 Mil.	3.1 Mil.
	Hematocrit	18	26	26			
	Hemoglobin (gm.%)	5.75	8.0	8.25	8.25	8.0	8.5
	Reticulocytes %		1.6			7.0	
	Leucocytes/cu.mm.	17000	16000	11000	10000	11000	9000
	PMN/Lymphocytes	53/43	72/28	63/32	53/44		53/40
	Eosinophils %	4	0	2	2		5
	Icteric index	40	68	60		12	
	Van den Bergh—						
	Indirect	12.4	12.3	5.59	3.42	3.08	
	Direct (mgm.%)	0.52	0.84	0.39	0.56	0.28	
Urine	Gross appearance & color	Cloudy & red	Cloudy & red	Clear & brown	Clear & amber	Clear & amber	Clear & amber
	Hemoglobinuria	4 plus	4 plus	0	0	0	0
	Urobilinogen	Pos. 1:1	Pos. 1:1	Pos. 1:5	0	0	0
	Albumin	1 plus	1 plus	0	0	0	0
	Sediment	Casts	Casts	0	0	0	0

During the first 24 hours hydration was maintained by oral and parenteral fluid administration. These fluids contained electrolytes in a combination designed to render the urine alkaline. Clinical jaundice increased during the first 16 hours of hospitalization. There was an associated rise of the serum icteric index from 40 to 68 units while the indirect van den Bergh remained at slightly over 12 mgm.%. During the succeeding 24 hours the indirect van den Bergh dropped to 5.59 mgm.% and the icteric index dropped slightly to 60 units. Hemoglobinuria ceased during that period. These data point to a cessation of hemolysis about 48 hours after the onset of jaundice. This was further shown by the stabilization of the hemoglobin at approximately 8 gm.% and the packed cell volume at 26% during that period without additional transfusions.

Laboratory data collected at the time of entry (see Chart I) were typical of an acute erythroclastic syndrome. There was a marked reduction of hemoglobin and packed cell volume, a high icteric index, and an elevated indirect van den Bergh determination, while the absence of urinary bile plus a continued normal stool indicated no biliary obstruction.

Peripheral blood studies revealed 1.6% reticulated red cells 24 hours after the onset of jaundice. Repeat studies done three days later showed a significant increase to 7.0%. Study of iliac crest bone marrow revealed accelerated erythropoietic activity. The marrow also contained an increased number of eosinophils as did specimens of peripheral blood. Follow-up examination two weeks after the onset of jaundice revealed four million R.B.C. per cu.mm. and 11 gm.% hemoglobin.

This depicted hemolytic syndrome occurring after ingestion of fava beans in a child potentially sensitized by previous ingestion was felt to be adequate grounds for a tentative diagnosis of favism.

Scratch tests carried out with antigen prepared from fresh fava beans were negative four days after the onset of jaundice. Such negative tests are commonly found for as long as six weeks following an acute attack.[4, 5] After this period the tests frequently become positive, as does the phenomenon of passive transfer.[4, 5] One American author, Lecks,[3] failed to demonstrate fava bean sensitization by these methods in the child he described, despite rather extensive studies carried out during several months succeeding the child's acute attack. The antigenicity of his skin test preparation could have been too low.

Repeat skin tests and passive transfer tests were negative when carried out two weeks after the onset of jaundice. Some insight into the possible immunological mechanisms of favism is being sought through additional experimentation currently in progress.

Summary

1. The clinical features of favism as it occurs in American children have been presented.

2. It is proposed that the surprisingly infrequent occurrence of favism in the United States may be explained by the lack of opportunity for pollen sensitization.

3. Favism occurring in a 28-month-old male child of Italian descent is described.

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STUDIES ON THE METABOLISM OF HUMAN CARDIAC MUSCLE OBTAINED BY AURICULAR APPENDECTOMY

Preliminary Report*

WALTER J. BURDETTE

In a previous report[4] on extirpation of the auricular appendage it was suggested that the cardiac muscle removed at such an operation could profitably be used for *in vitro* metabolic studies. Since that time cardiac muscle removed at the time of auricular appendectomy in two patients has been used for this purpose, and results of these studies are reported here.**

Methods†

The auricular appendage was removed from the right side in both patients. Other than arteriosclerosis in both cases and left axis deviation in the second case no abnormality of the cardiovascular system was noted before operation. Both patients had carcinoma of the lung, and an exploratory thoracotomy was done in the hope that a resectable lesion would be found. In each case the lesion was too extensive to permit resection, the pericardium was opened, and in a few minutes the appendage was removed before the thoracotomy wound was closed. An old pericarditis was encountered in the first case. In neither case was there any untoward effect of the operation. The first patient died from extension of the neoplasm one month later after receiving nitrogen mustard therapy. The second patient is now receiving palliative treatment after recovering satisfactorily from the operation.

Immediately after the muscle was removed it was placed in a petri dish resting in ice in order to prevent depression of respiration from anoxia.[8] Slices of the tissue 0.5 mm. in thickness were quickly made, and oxygen consumption was measured by the direct method of Warburg, using 100% oxygen as the gas phase. Readings were taken at intervals of 15 minutes. In addition to Krebs-Henseleit physiological saline buffered with phosphate[10] containing half the original concentration of calcium, substrates were added at zero time after the vessels had been equilibrated for 10 minutes to make the following final concentrations: 0.02 M sodium pyruvate, 1.0 mM glucose, 2.0 mM sodium iodoacetate, and 0.01 mg. % lanatoside C.‡ For one of the samples the concentration of potassium was 0.4 mM rather than the usual 5.0 mM potassium concentration of Krebs-Henseleit physiological saline. The respiratory quotient of the tissue was determined in the second case by the second method of Dickens and Simer,[7]

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** In the course of these studies the auricular appendage has now been removed from five patients without deleterious effect. The operation is technically not difficult, but it seems indicated as a biopsy only when a thoracotomy is done for some other reason. The muscle has been obtained either when it was removed in the surgical treatment of cardiac disease or when a malignant lesion was found which was not resectable. Whether sufficient information may be obtained from the excised muscle to justify operation for that purpose alone will rest on further observations.

† The technical assistance of Mr. Bryan Harwell is gratefully acknowledged.

‡ Supplied through the courtesy of Sandoz Pharmaceuticals.

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using the Dixon-Keilin type of vessels. The results are given in Tables 1 and 2 where oxygen consumption is expressed as cu.mm. of oxygen consumed per mg. dry weight of the tissue per hour.

Results

In the second case the Q_{O_2} values varied from 6.39 to 2.28 during the first three hours when Krebs-Henseleit buffer was present alone; in the first case, however, the corresponding values were much lower, ranging

Table 1

Respiration of Human Cardiac Muscle
(Case 1)

	No. of determinations	Q_{O_2}					
		30'	*60'*	*90'*	*120'*	*150'*	*180'*
Saline	1	0.49	0.98	1.23	0.74	0.98	1.23
Pyruvate	1	5.57	4.51	4.51	3.98	3.98	3.18
Iodoacetate	1	2.42	0.66	1.32	0.44	0.44	0.44
Saline (0.4mM K)	1	4.30	2.15	2.80	1.72	1.72	1.08

Table 2

Respiration of Human Cardiac Muscle
(Case 2)

	No. of determinations	Q_{O_2}					
		30'	*60'*	*90'*	*120'*	*150'*	*180'*
Saline	2	6.39	3.21	2.28	2.94	3.22	3.07
Pyruvate	1	10.83	5.99	3.42	5.13	3.99	5.42
Glucose	1	9.30	4.80	2.10	3.60	2.60	3.40
Iodoacetate	1	3.08	1.63	0.97	.00	.00	.00
Lanatoside C	1	9.18	12.49	13.26	9.44	9.44	5.35

from 1.23 to 0.49. When 0.4 mM potassium was present in the saline in the first case, the initial level was higher, but it declined from 4.30 to 1.08 in three hours. The average dry weight was found to be 15.2% of the weight of the wet samples.

Addition of sodium pyruvate resulted in an increased oxygen uptake in both cases, reaching levels as high as 5.57 in the first and 10.83 in the second. Only 7.2×10^{-5} M pyruvic acid was present at the conclusion of the experiment. When glucose was added the Q_{O_2} determination was 9.30 at the beginning and 3.40 at the end of three hours (Table 2). The presence of lanatoside C had a pronounced effect on oxygen uptake, the Q_{O_2} rising as high as 13.26 in the vessel containing it.

The Q_{O_2} levels were fairly well sustained after 90 minutes in all of the vessels except those containing sodium iodoacetate. This substrate caused rapid decrement in oxygen consumption reaching negligible proportions after 90 minutes. The respiratory quotient was found to be 0.96.

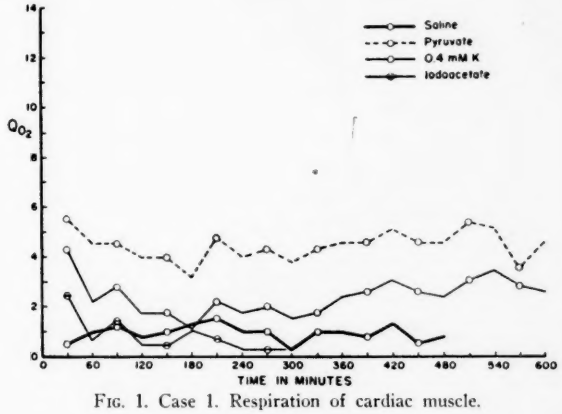

FIG. 1. Case 1. Respiration of cardiac muscle.

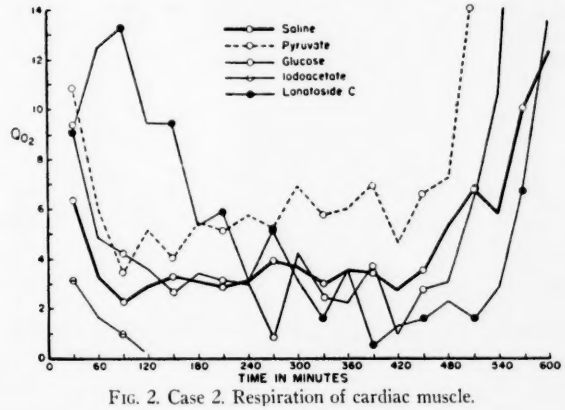

FIG. 2. Case 2. Respiration of cardiac muscle.

Curves for Q_{O_2} levels for Case 1 are presented in Figure 1, those for Case 2 in Figure 2. With two exceptions (saline in Case 1 and lanatoside C in Case 2) there was an initial decrement during the first hour. This decline continued when the muscle was poisoned with iodoacetate. In Case 2 the presence of lanatoside C was associated with an initial rise in Q_{O_2} followed by a fall after 90 minutes. This continued to a point below the level obtained with physiological saline and remained there after five hours.

The shape of the curves when pyruvate was added was very similar to that when saline was present alone except that the Q_{O_2} values were higher with pyruvate. The initial Q_{O_2} for the vessel containing glucose was at a higher level (than when no substrate was present) for only two hours.

Beginning about the seventh hour there was a marked rise in oxygen consumption in all vessels used for Case 2 (except the one containing sodium iodoacetate which was not observed after three hours). This increased respiration was observed until readings were discontinued at the end of twelve hours. Cultures taken at that time were positive for *Alcaligenes faecalis* and *Staphylococcus albus*. Subsequent inoculation of Warburg vessels with strains of these organisms under the same conditions, except for the addition of cardiac muscle, resulted in similar changes in manometer readings, although of lesser magnitude. This occurred after six hours, and it is assumed that the late changes in manometer readings described are attributable to the presence of these organisms.

Discussion*

Probably more reliance may be placed in the results reported for the second case than those of the first case where pericardial adhesions were present, fewer determinations were done, and where the Q_{O_2} with 0.4 mM potassium was higher than with 5.0 mM potassium. The Q_{O_2} values for human muscle are somewhat lower than similar values in experimental animals from this and other laboratories,[1, 16, 18] especially the low Q_{O_2} determinations in Case 1 when physiological saline was present alone.

The presence of sodium pyruvate was clearly associated with a pronounced and sustained elevation of oxygen consumption. This was not a non-specific effect, since pyruvate actually disappeared from the medium. There is general agreement that pyruvate initiates an increase in oxygen consumption in experimental animals,[1, 2, 5, 14, 15, 17, 18] and Goodale, *et al.*[5] have reported lower concentrations of pyruvate in human coronary venous blood than in arterial blood.

The proximity of the respiratory quotient (0.96) to unity, an indication of the metabolism of carbohydrate, is additional evidence for the importance of carbohydrate utilization by the tissue which was studied. However, this R.Q. is considerably higher than the value found by Powers and Bing[3] who made determinations on coronary blood.

The findings with glucose should be interpreted cautiously,[11] particularly in view of previous experience[5] and that of others[1, 2] in failing to find an increase in Q_{O_2} with glucose substrate in the rat. Although initially the determinations were elevated, the remainder of the readings showed little change from the control. It should be noted, however, that numerous authors have shown that glucose can be used for the energy of contraction

* Suggestions of Dr. A. E. Wilhelmi during preparation of the manuscript were very helpful.

under certain conditions.[6, 13, 17] The effect of lowering the concentration of potassium may likewise be considered equivocal (Table 1).

Sodium iodoacetate, which inhibits oxidation and reduction, effectively blocked the respiration of the slices. The importance of phosphocreatine in transferring chemical energy to muscular work has been inferred by several workers;[8, 9, 12] the results obtained with sodium iodoacetate may therefore mean that no energy is available for the resynthesis of phosphocreatine due to inability to utilize sugar because of inhibition of the oxidative phosphorylation of phosphoglyceraldehyde to phospho-glyceryl-phosphate. However, this is not conclusive evidence that the phosphocreatine cycle is operating, since iodoacetate inhibits a number of enzyme systems.

The increase in Q_{O_2} following contact with lanatoside C is impressive although it was not prolonged much beyond the fourth hour. The effect of the glycoside on respiration of the slices, of course, is not necessarily related to inotropic effects of the drug.[18] It should also be noted that the effect described was obtained without addition of substrate, which suggests that this drug acts in some manner other than by facilitating entrance of exogenous substrate into the cell.[18] The danger of drawing hasty conclusions from a single observation deters further speculation.

The data presented are necessarily scanty because of the limited source of the tissue, and, even if the number of samples were of desirable magnitude, the chemical events during contraction could scarcely be analyzed accurately from the information. The extent to which results using the auricle may apply to ventricular muscle also awaits further study. Of more importance than the data presented is the continued use of cardiac biopsy for study of the metabolism of human cardiac muscle. Through the use of this method it should be possible to learn about the normal metabolic processes involved in contraction and their alteration in disease. The relation of phosphate bonds to energy transfers is of particular interest. Also the combination of the method described here with the use of labelled compounds and with catheterization of the coronary sinus appears especially promising. There are certain problems which ultimately can be solved accurately only in human tissue, and confirmatory evidence in man of the findings in experimental animals is always desirable.

The data presented are compatible with the idea that the muscle tested was respiring aerobically, utilizing carbohydrate with adequate metabolic reserve to respond to additional pyruvate and to the presence of lanatoside C, respectively, with increased Q_{O_2}, and that the tricarboxylic and phosphocreatine cycles are operative in the intermediary metabolism of the tissue. Further studies are desirable in order to verify and extend these observations.

Summary

1. Slices of human cardiac muscle which were obtained by excising the auricular appendage in two patients were studied in the Warburg apparatus.

2. The presence of sodium pyruvate resulted in an increased Q_{O_2}.

3. Lanatoside C in the absence of exogenous substrate caused the greatest increase in oxygen consumption of the tissue slices.

4. Sodium iodoacetate blocked the respiration of the muscle.

5. The respiratory quotient was found to be 0.96.

6. The use of cardiac biopsy for obtaining and studying fresh cardiac muscle from the living patient should be a valuable adjunct to the study of human cardiac physiology in health and disease.

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MORTALITY DUE TO RHEUMATIC HEART DISEASE IN THE SOCIO-ECONOMIC DISTRICTS OF NEW HAVEN, CONNECTICUT*

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A vast amount of work by Sydenstricker and others, on the effect of environment on health in the United States, has followed the earlier work of Gottwald, René Sand, Newsholme, and Ramazini along similar lines in Europe. Correlation of morbidity and mortality rates for human disease with the socio-economic conditions of the group involved is both an important and an extremely complex problem. This type of study is a part of the broader field of endeavor which has been variously termed comprehensive medicine, the social pathology of disease, and the ecology of human disease.[a] An outstanding proponent of this concept has been the late Dr. John A. Ryle, Director of the Institute of Social Medicine at Oxford University.

Computing morbidity or mortality trends for certain ecological areas within a community usually necessitates a new appraisal of the true socio-economic divisions since old conceptions of dividing lines based on artificial boundaries or political wards, or casual observation of the "good" and "bad" districts cannot be relied upon to give an accurate picture of the community. Fortunately, in New Haven, Connecticut, the site of this study, Davie[z] had made a thorough and extensive socio-economic study based on information collected during the years 1933-1935. This survey was much more than a study of economic levels in that it was related to family income, race, delinquency, and dependency, as well as inclusion in the *Social Register, Who's Who,* and membership in the local Graduate Club. Sheps and Watkins[s] combined the 25 small subdivisions which Davie outlined into three major areas for a mortality study of the city during the years 1930-1934. *Major area 1,* the uppermost stratum, comprised the two best residential sections containing a large proportion of the "notables" in the city listed in the *Social Register, Who's Who,* and the local Graduate Club roster. The family heads were principally business executives, professional persons, dealers, proprietors, and office workers. Single family dwellings of relatively high evaluation and two-family houses predominated. The family income ranged upwards from a low of between $1,500 and $3,000

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per year, with a considerable portion above $3,000. The majority of family heads were native-born. There was no delinquency and practically no problem of dependency. *Major area 2* was composed of three middle strata. These areas were predominantly residential but contained some business, industrial, and commercial activity. Artisans, office workers, and laborers predominated as family heads. Two-family houses were the rule. Family incomes ranged from a few which were above $1,500 to the majority below $1,500 annually. From one-third to one-half of the family heads were foreign-born. Delinquency and dependency were generally below expectancy. *Major area 3* was made up of two "blighted" and slum areas with commerce and industry scattered throughout. Housing in one area was generally below desirable and healthful standards, when measured by physical characteristics, and in the other area consisted of crowded two, three, and multi-family units of the poorest quality. Over eighty per cent of family incomes were below $1,500 yearly and a significant proportion was below $1,000. Predominant occupations of family heads were artisans or laborers. These areas contained the highest proportions of foreign-born family heads. Delinquency and dependency rates were very high. This area contained the Negro neighborhood. By detailed work on population figures, oftentimes necessitating house-by-house and block-by-block analysis of the number living in dwelling units, they calculated population figures for the major areas.[8] Using the basic data of Sheps and Watkins, an attempt has been made in this study to determine any changes in the death rates (due to rheumatic heart disease) which occurred in each of the three major ecological areas for the periods 1920-1929, 1930-1939, and 1940-1948.

The total population of the city of New Haven has not changed much since 1920 but the relative age groups have changed in a manner similar to that for the United States as a whole; i.e., the per cent of population in the younger age groups including the first three decades is diminishing, whereas the per cent in the older age groups—5th, 6th, 7th, and 8th decades —is increasing. In regard to the population in the ecological areas, it would be highly desirable to know the exact population for each area for each year of the study, but this information was available only for the years 1933-1935. It was the opinion of Davie that there have been a number of local changes in the distribution of population and character of areas since his ecological study was made in 1933-1935, but if the three major areas of Sheps and Watkins were used in the present study, the over-all picture concerning distribution of population would not have changed significantly.[1] Concerning housing conditions in New Haven, there have been no actual field surveys since the 1920's. But according to Dr. C.-E. A. Winslow[9] "The only change which has taken place in the city itself is the erection of 1,600 units of low-rent and moderate-rent housing. This, of course, has improved the situation to some extent. About half of these units were built on vacant land and the other half represent substantial clearance of two

of the worst areas in the city. Beyond this it is doubtful if any substantial changes have occurred."

METHODS

The total number of deaths due to rheumatic heart disease in New Haven from 1920-1948 was determined after careful study of each death certificate located in the Office of Vital Statistics for the City of New Haven.* Deaths attributed to rheumatic heart disease or one of its complications were listed under the nine categories below:

1. Rheumatic heart disease with heart failure, 25 years of age and over.
2. Acute rheumatic heart disease with heart failure occurring in the age period 2 to 25 years.†
3. Rheumatic heart disease plus bacterial endocarditis where the latter was the immediate cause of death.

TABLE 1

POPULATION DATA FOR NEW HAVEN, CONNECTICUT
1920, 1930, 1940, AND 1950

Year	*Total Population*	*2-24 years***	*Over 24 years*
1950	163,344	61,053	95,968
1940	160,605	60,162	96,739
1930	162,655	69,316	88,521
1920	162,537	69,176	86,327

Source: 14th, 15th, and 16th Census of the United States and the 1950 census of population—preliminary counts.

** Estimated by deducting twice the enumerated population under one year from the enumerated population under 25 years.

4. Rheumatic heart disease plus other conditions contributory to the cause of death.
5. Other immediate causes of death plus rheumatic heart disease.
6. Rheumatic heart disease plus pregnancy where the death was due to the combination of the two.
7. Immediate cause of death, cerebral embolism plus rheumatic heart disease where the source of the embolus was the heart; no recent myocardial infarction.
8. Immediate cause of death, pulmonary embolism plus rheumatic heart disease where the source of embolus was the heart; no recent myocardial infarction.
9. Immediate cause of death, embolism to other organs or areas plus rheumatic heart disease where the source of the embolus was the heart; no recent myocardial infarction.

* It is a pleasure to express appreciation to the Office of Vital Statistics of the City of New Haven which made available the original death certificates. Particularly helpful were Mr. John T. Coleman, Registrar, and Miss Anna V. Daly, Assistant Registrar.

† The arbitrary division into the two age groups, 2 to 25 years, and 25 years and older, was made because heart failure in these two age groups occurs for different reasons; i.e., in the age group 2 to 25 years heart failure and death are usually due to active rheumatic heart disease, while in the older age group (25 years and older) heart failure is usually not due to active rheumatic heart disease.

Addresses of the deceased at the time of death were obtained from the death certificates and then located in one of the three major ecological areas established by Sheps and Watkins and based on the work of Davie.

TABLE 2

NUMBER OF DEATHS IN EACH OF THE ECOLOGICAL STRATA

	Year	*Stratum 1*		*Stratum 2*		*Stratum 3*	
	1948	2		12		9	
	1947	6		6		10	
	1946	2		4		19	
	1945	13	Pop.*	4	Pop.	17	Pop.
	1944	7	34,592	13	43,362	15	84,451
	1943	4		15		21	
	1942	7		12		21	
	1941	10		7		16	
	1940	2		8		30	
Total for (1940-1948)		(53)—18.2%		(53)—27.7%		(158)—54.1%	
	1939	5		17		19	
	1938	4		9		30	
	1937	9		7		22	
	1936	6	Pop.	8	Pop.	29	Pop.
	1935	7	34,427	6	43,155	23	84,048
	1934	4		10		29	
	1933	6		9		18	
	1932	6		10		28	
	1931	3		4		29	
	1930	5		15		28	
Total for (1930-1939)		(55)—13.5%		(95)—23.5%		(255)—62.9%	
	1929	5		7		14	
	1928	8		11		24	
	1927	2		9		22	
	1926	4	Pop.	7	Pop.	29	Pop.
	1925	2	34,633	8	43,413	32	84,551
	1924	0		12		36	
	1923	4		8		23	
	1922	5		8		25	
	1921	3		8		31	
	1920	2		11		31	
Total for (1920-1929)		(35)—8.9%		(89)—22.8%		(267)—68.3%	
Total (1920-1948)		(143)—13.1%		(265)—24.3%		(680)—62.6%	

* Pop. = population estimate for each stratum.

Crude annual age specific mortality rates in each of the three strata were calculated in the usual manner for the age groups 2-25 years, and 25 years and older. In the absence of exact population figures for each year, a graph was constructed showing population trends for the two age groups (2-24, and 25 or more years). Estimates of the population for the two age groups for each year were obtained from the graph and used for calculating annual age specific rates. Population figures used in the construction of the graph and determination of rates are shown in Table 1.

Results

A total of 1,144 deaths due to rheumatic heart disease or one of its complications occurred in the city of New Haven from 1920 to 1948. It was possible to determine for 1,088 individuals the stratum in which they were

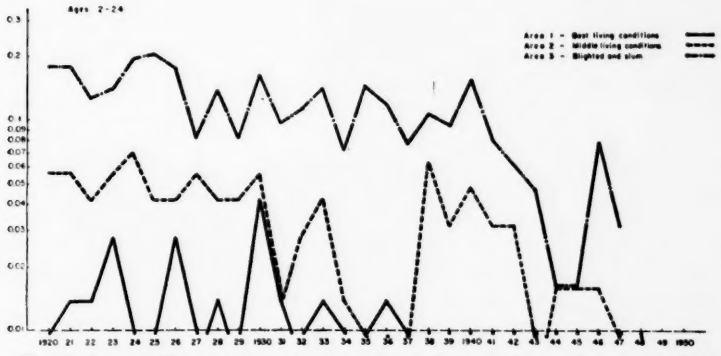

FIG. 1. Age specific mortality rates from rheumatic heart disease (age group 2-24 years) in three different socio-economic areas within the city of New Haven 1920-1948.

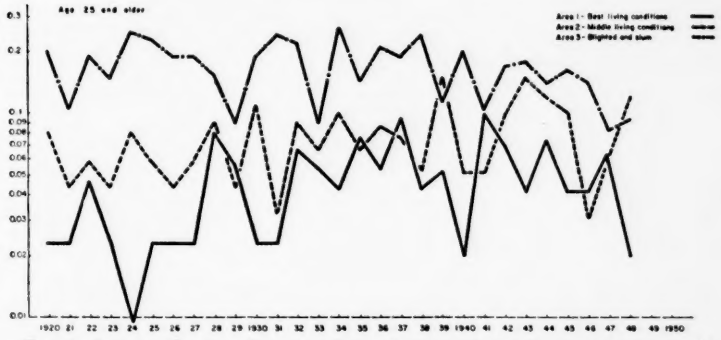

FIG. 2. Age specific mortality rates from rheumatic heart disease (age group 25 years and older) in the three different socio-economic areas within the city of New Haven 1920-1948.

living at the time of death. Some death certificates did not have complete information on addresses, and a few had illegible street addresses. In Table 2 is shown the number of deaths of individuals for each stratum by year from 1920 to 1940. The total number of deaths for three periods, 1920-1929, 1930-1939, and 1940-1948 are recorded along with the per cent of the total number of deaths which occurred in each stratum for each of the three periods 1920-1929, 1930-1939, and 1940-1948. Also shown are the average

population figures of each stratum for each of the three periods. During the years 1933 to 1935, 21.3% of the city's population lived in the best stratum (area 1), 26.7% in the middle stratum (area 2), and 52.0% in the worst stratum (area 3), a ratio of roughly 1:1:2, respectively. Between the years 1920-1948, 13.1% of the 1,088 deaths occurred in individuals who had lived in the best stratum, 24.3% in the middle stratum, and 62.6% in the worst. Throughout the 28-year period the populations in the three strata were nearly constant in number, yet the percentage of deaths in individuals living in the best areas (stratum 1) increased from 8.9% to 18.2%, whereas the percentage of deaths occurring in the worst areas decreased from 68.3% to 54.1%.

The age specific mortality rates shown in Figures 1 and 2 indicate that the rates were consistently higher in both age groups (2-24, and 25 and older) in the blighted or slum area (major area 3) than in the middle (major area 2) and best (major area 1) ecological areas. The rates in the middle stratum were consistently higher than in the best areas. Age specific death rates for the age group 2-24 years show a definite downward trend, most marked in the poorest living area but also definite in the middle area. It is difficult to see any downward trend in the best ecological area because the number of deaths was too small, but it is noteworthy that no deaths occurred in this area in the age group 2-24 years between 1936 and 1948.

In the age group 25 years and older there did not seem to be any definite trend either up or down in death rates in either of the three ecological areas unless the slight increase in rates in major area 1 (best ecological area) could be called a trend upward.

Discussion

The results indicate that the mortality rates due to rheumatic heart disease in New Haven during the years 1920-1948 have been consistently higher in the poorest living area than in the middle or best strata. These findings are consistent with the concept that rheumatic fever rates are usually highest in an environment where crowding is most prevalent, and housing and economic status the lowest. It would seem that in New Haven, continued residence in the poorest living areas after the onset of rheumatic fever would carry a greater risk of mortality due to rheumatic heart disease than continued living in the two better ecological areas. Whether this might be due to environmental conditions or the quality and quantity of medical care, or a combination of the two, or to other less obvious reasons remains to be investigated. The recent report of Knowleden[5] from England and Wales noted a moderate degree of association between the death rate due to rheumatic heart disease and the proportion of employed males belonging to the lowest social classes. Our data also show that death rates due to rheumatic heart disease in the age group 2-24 years are decreasing in

each of the three ecological areas. The general decrease in mortality due to rheumatic heart disease in New Haven confirms the observation of others, both in this country and England, that rheumatic fever and rheumatic heart disease rates have been declining for 30 years or more[3, 4, 10] although the only downward trend in this study group was in the age group 2-24 years, but not in the age group 25 years and older. These findings have been described in more detail in a previous study[7] which indicated that in New Haven the death rates due to *active* rheumatic heart disease since 1920, i.e., deaths below the age of 25, are decreasing, but the death rates due to rheumatic heart disease in age groups older than 25 have not changed since 1920.

Summary and Conclusions

1. Mortality rates due to rheumatic heart disease in the city of New Haven, Connecticut, from the year 1920 to 1948 inclusive have been related to the three major ecological divisions of the city.

2. During the years covered by this study, there were consistently higher death rates due to rheumatic heart disease in the poorest living areas than the middle or best living zones.

3. A distinct trend was noted: namely, that mortality rates due to rheumatic heart disease in the age group 2-24 years appear to be decreasing in both the poorest living area and in the middle ecological area.

4. No distinct changes in death rates due to rheumatic heart disease could be seen in the age group 25 years or older in any of these ecological areas.

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DAILY VARIATIONS OF STANDING POTENTIAL DIFFERENCES IN HUMAN SUBJECTS*

Preliminary Report

LEONARD J. RAVITZ†

Biological and psychological sciences have tended to lag far behind physics in their approach to the nature of design in living systems. To test the hypothesis that protoplasmic systems might also exhibit charges and field forces responding in an orderly fashion to the same physical laws controlling the inorganic universe, Burr perfected a microvoltmeter, with the assistance of Lane and Nims, by means of which he could study basic electrical properties of living creatures.[6, 7, 8] Experiments of the past twenty years have shown unequivocally that in all living membranes, tissues, cells, and organisms, there is a well-defined, relatively steady, stable voltage gradient of considerable magnitude between any two points—altered only by biological changes in the organism—which is characteristic of the species and, to some extent, characteristic of the individual.[1, 6, 9] Lund's experiments furnish additional evidence for the existence of electrical fields and indicate that inherent electrical forces are important determinants of polarity and of organization in all living systems.[10]

To ascertain whether humans evidenced any day-to-day EMF alterations, DC spot determinations were taken daily from two to eight months on ten subjects, seven men and three women. All except one were university students. The 3A5 DC-powered microvoltmeter was used which, employing essentially a Wheatstone bridge circuit, with a 10 megohm input impedance, high sensitivity, and high stability, measures DC potentials as pure voltage gradients independent of current and resistance changes. Reversible, nonpolarizing Ag-AgC1 electrodes immersed in physiological saline were brought into contact with the subjects. To wash out any errors in single determinations, a bilateral series of four potential differences between head and anterior chest wall were summated:

Grid electrode		*Ground electrode*
Right temporal region	—1—	right chest, midclavicular line
Right temporal region	—2—	left chest, midclavicular line
Left temporal region	—3—	right chest, midclavicular line
Left temporal region	—4—	left chest, midclavicular line

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Two such series were taken at each reading, and the results were averaged both as absolute values and with signs. Care was taken that no metal contacted the individuals; electrode drift, balance, sensitivity, and the possibil-

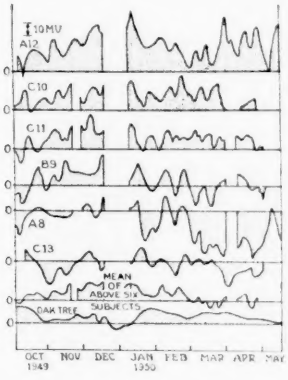

FIG. 1. Five-day running averages of mean daily PD's with signs, from two sets of four readings each for six subjects and the oak tree.

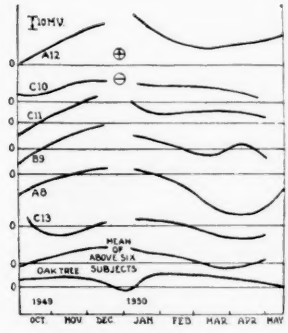

FIG. 2. Eight-month PD trends with signs, for six subjects and the oak tree.

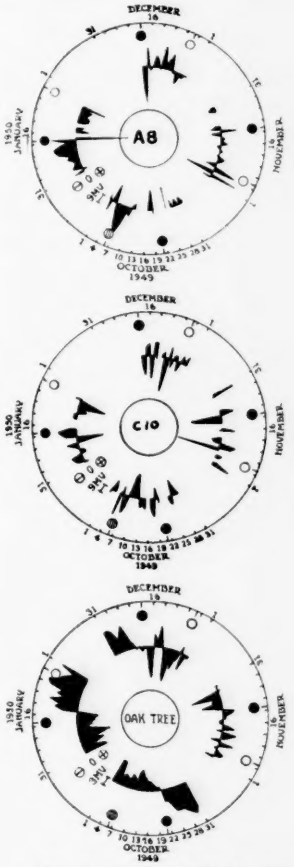

FIG. 3. Sample algebraic polar coordinate plots showing deviations of daily mean from monthly mean PD during different moon phases, for two subjects and the oak tree.

ity of air bubbles in the electrodes were constantly checked. Electrodes were reversed at least every third or fourth reading. Notation was made of local variations in the external environment, e.g., temperature, barometric pressure, humidity, weather, and sunlight, and of body temperature, blood

pressure, and pulse of the subjects. Results in millivolts were plotted with and without signs as five-day running averages, deviations of the daily mean from the monthly mean, monthly means, and as monthly ranges (the difference between the highest positive and highest negative daily averages for the month).

In all subjects, 14- to 17-day cycles were present which roughly correlated with lunar phases (Fig. 1). In addition, there was evidence to indicate four-month cycles, and in one individual, six-week cycles. As these phenomena varied somewhat among the subjects, combined five-day running averages of the six subjects measured for the longest period of time were plotted. These averages maintained approximately the same cyclic pattern seen in individuals (Fig. 1), which, as in individuals, was more marked when polarity was taken into account. These are similar to tree cycles,[2, 4, 5] although they show greater voltage fluctuations and decreased polarity stability. As with older trees,[5] voltage fluctuations were relatively minimal in a middle-aged subject, C-13 (who also showed six-week cycles). No apparent correlation existed between DC measurements and temperature, blood pressure, and pulse,* or with local environmental variations. The possibility of recurring instrument artifact was ruled out by attaching electrodes immersed in a column of saline to a microvoltmeter and taking continuous readings for several months without any potential change.

There was a definite long-range tendency for most subjects to increase in positivity (i.e., head positive to chest) from October through the middle of December, 1949, and to decrease in positivity from the middle of January to May, 1950 (Fig. 2). The head was usually positive or slightly negative to the chest; high negative readings were less frequent than high positive readings. The normal monthly mean was roughly —7 to +18 millivolts, although on occasion it deviated as widely as —29.8 and +34.9 millivolts. The highest daily averages were —70.5 and +69.5 millivolts.

Other than approximate periodicity with the full or new moons (a marked PD increase or decrease occurred at these times, although marked daily deviation from the monthly mean more frequently preceded or followed the lunar day by 24 to 72 hours), the only other correlation with daily DC measurements seemed to be with disturbed emotional states, which for the most part could not be associated with high isolated PD readings, but only with sustained high PD's exceeding —15 or +25 millivolts. Although the cycles appear more significant when polarity is taken into account, monthly means correlate better with emotional disturbances when determined as absolute values, rather than with signs. Deviations of the daily mean from the monthly mean often appeared similar to tree deviations (Fig. 3). In many instances the human and the tree potential differences

*Skin temperature differences modify standing potentials in the ratio of 2.5° C./millivolt.[3]

were mirror images. Further studies now in progress at Duke and at the Veterans Administration Hospital, Roanoke, Virginia, reveal the same rhythmic variations.

A report of correlations with emotional states will be published later.

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PHEOCHROMOCYTOMA*

DANIEL B. ARST, WILLIAM J. LAHEY, AND PAUL KUNKEL

In recent years, pheochromocytoma has been found to occur much more frequently than was previously suspected, and the importance of early recognition of this tumor has been stressed by numerous authors.[1-3, 18] This report of two cases of pheochromocytoma presents interesting clinical observations with particular reference to physiological and pharmacological factors.

Case Reports

Case #1. R. T., a 24-year-old white male, was admitted February 11, 1947 because of visual disturbances. He had had no serious illnesses in the past. At no time was hypertension noted until his discharge from the service in October, 1945, when the patient was told that his blood pressure was "a little high."

He consulted a physician on two occasions for minor illnesses in 1946, and on both occasions the blood pressure was elevated to "around 185." Eight months prior to admission the patient noted the onset of a dull frontal headache, which occurred in the morning on arising and after exercise and was relieved by aspirin. Ten days prior to admission, the patient noticed sudden blurring of vision, followed three days later by complete loss of vision in the right eye.

Physical examination on admission revealed marked changes in both fundi (old and recent hemorrhages, extensive scarring with involvement of the right macula, arteriovenous nicking, and bilateral papilledema), and a blood pressure of 180 mm. Hg systolic and 118 mm. diastolic. The heart size was within normal limits.

Pertinent data are presented in Table 1. Intravenous pyelogram was normal. During the first few days in the hospital, a new hemorrhage in the region of the left macula appeared and was followed by increased papilledema and further loss of vision. In view of the patient's rapidly progressive disease it was decided to explore the adrenal areas, and, if no tumor were found, to perform a sympathectomy.

On the ninth hospital day the patient was operated upon. Shortly after removal of the right ninth rib the patient developed pulmonary edema and the procedure was discontinued. Despite supportive measures, his condition became progressively worse and he died on the fourth postoperative day. Postoperatively, the blood pressure remained at high levels, varying between 170/150 and 240/179 mm. Hg.

Autopsy revealed a tumor measuring 10 x 5 x 3 cm. and weighing 314 grams, lying above and anterior to the upper pole of the right kidney. It was intimately adherent to the inferior vena cava over an area approximately 4 cm. in length, in the center of which was a large venous connection leading from the tumor. Grossly the mass appeared to be composed of two types of tissue. Histologically, it had the characteristic appearance of pheochromocytoma.

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Two samples of the tumor were analyzed.* One, weighing 299 mg., contained 64 micrograms per gram of a substance which appeared colorimetrically to be identical with epinephrine. The other, weighing 229 mg., contained 140 micrograms per gram of catechol compounds closely related to but not identical with epinephrine.

TABLE 1

PERTINENT DATA IN CASE 1 (R.T.) AND CASE 2 (G.L.).

		G.L.	
Patient	*R.T.*	*Pre-op.*	*Post-op.*
Symptoms & Signs			
Hypertension			
Sustained	+	+	+
Paroxysmal	—	+	—
Headache	+	+	±
Visual disturbances	+	—	—
Hyperthermia	—	+	—
Hyperhidrosis	—	+	—
Eyeground changes	Gr. IV	Gr. I	Gr. I
Cardiac enlargement	—	±	±
"Average" blood pressure	200/140	190/130	140/110
Laboratory data			
Urine	+, ++ alb.	Occ. + alb.	—
Fasting blood sugar	139 mgm. %	156	
I.V. Glucose tolerance (Fasting/1 Hr./2 Hr.)	Not done	160/205/130	75/85/80
Basal metabolic rate	Not done	+42, +47, +37	—1, +4
EKG changes	—	+	+ → —
Functional tests			
Sedation	Neg.	Neg.	Neg.
Cold pressor	Hyper. response	Normal	Normal
Postural changes B.P./Pulse	0/+	—/+	+/+
Histamine	Not done	+	—
Benzodioxane	Not done	+	—
Dibenamine	Not done	+	—
Massage adrenal areas	—	—	—

Most postoperative data for G.L. were recorded shortly after operation. The sedation test was unchanged at that time but became positive seven months after operation.

Discussion. This patient illustrates the importance of early recognition of this entity and the ease with which it can be confused with other types of hypertension. The presence of hypertension in a young man should have

* We wish to think Dr. W. Raab of the University of Vermont College of Medicine for the analysis. According to him, the specific amounts must be regarded with caution in view of the time elapsed between obtaining the specimens and their receipt at his laboratory.

stimulated further search for a specific cause at the outset. A significant number of cases will be missed if paroxysmal hypertension is considered a prerequisite for diagnosis. The patient's reaction to the operative procedure weighted the evidence in favor of a pheochromocytoma sufficiently to allow the diagnosis to be made ante-mortem. In view of the newer knowledge relative to epinephrine and arterenol (nor-epinephrine), it is possible that the latter comprised a significant portion of the pressor amines found in the tumor.

Case #2. G. L., a 32-year-old white male cook, entered this hospital on December 17, 1949 with complaints of headache, dizziness, and excessive perspiration. Symptoms first occurred in 1945 following combat on Okinawa. The blood pressure was found to be high and, after several months of hospitalization, he was discharged from the service with a diagnosis of hypertension.

In July, 1946 he was treated at home with bed rest for nineteen weeks because of fever and migratory polyarthritis, which was diagnosed as rheumatic fever. Later that year his headache recurred, became more severe and was accompanied by dizziness. He was hospitalized for two weeks and discharged with the diagnosis of essential hypertension.

In the year prior to admission the patient continued to have episodes of severe headache, dizziness, palpitation, and sweating. He also noted some shortness of breath on exertion and a sense of increased warmth. He became increasingly irritable and finally stopped work in April, 1949. Since the symptoms continued to progress despite treatment, hospitalization was advised.

The past history was not remarkable. His mother had died of "high blood pressure." The patient was married and the father of a young child. There were emotional difficulties in his family situation, the nature of which could not be clarified.

On admission, the temperature was 99° F., the heart rate 100, the respiratory rate 16, blood pressure 200 mm. Hg systolic and 154 mm. Hg diastolic. The patient was a well-developed, well-nourished, white male, who appeared apprehensive, but presented no overt evidence of acute or chronic illness. Physical examination was not remarkable except for the hypertension, grade I hypertensive retinopathy, and evidence of peripheral arteriosclerosis.

Pertinent data are presented in Table 1. Of particular interest are the persistently elevated basal metabolic rate and a reduced glucose tolerance. Renal function studies were normal. Electrocardiogram was within normal limits and the chest x-ray showed slight left ventricular prominence. Intravenous and retrograde pyelograms showed a persistent filling defect, interpreted as a cyst, in the upper pole of the left kidney.

The patient ran a low-grade fever and persistent hypertension during his preoperative course. There were paroxysms of sweating, headache, palpitation, and dizziness, during which the blood pressure rose to 250/160 mm. Hg. At all times the patient complained of increased warmth and preferred to sleep near an open window.

Following the intravenous administration of 0.025 mg. of histamine phosphate, the blood pressure rose from a resting level of 200/130 mm. Hg to 280/170 mm. Hg, and the patient experienced symptoms similar to those which occurred during a spontaneous paroxysm. The blood pressure remained elevated over a six-minute period and then fell to pre-injection levels in approximately 25 minutes.

The preoperative benzodioxane and Dibenamine* tests are shown in Figures 1 and 2.

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* Kindly supplied by Smith, Kline and French Laboratories.

The studies strongly supported the diagnosis of a pheochromocytoma and operation was planned. However, an abnormal electrocardiogram was noted on January 27, 1950 and on January 30 the patient complained of joint pains. These were never severe, nor were there any objective changes. The temperature rose slightly and there

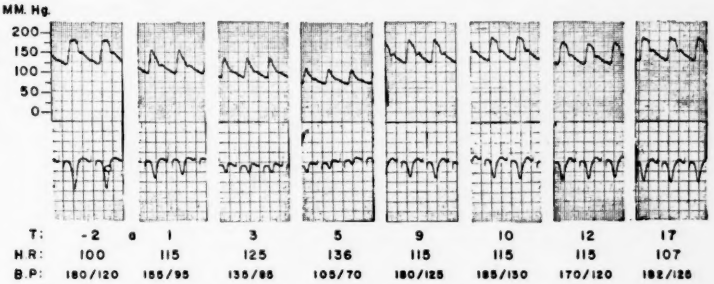

FIG. 1. Case 2. Tracings recorded with Sanborn electromanometer and Poly-viso Cardiette.

Pheochromocytoma: effect of intravenous administration of 20.0 mgm. piperoxane hydrochloride.

Upper tracing—brachial artery pressure; lower tracing—EKG, lead V_4; a—Injection of piperoxane (over two-minute period); T—time in minutes from beginning of injection; H.R.—heart rate; B.P.—blood pressure.

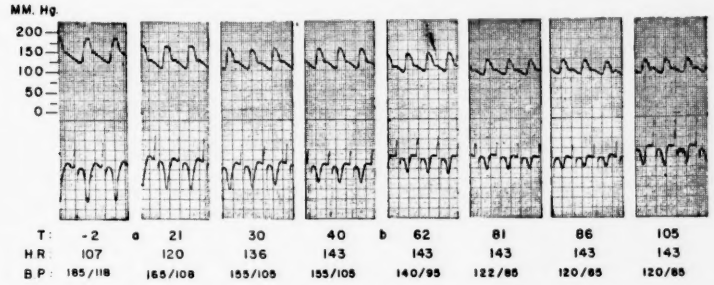

FIG. 2. Case 2. Preoperative Dibenamine test, (300 mgm. of Dibenamine HCl given intravenously). Tracings recorded as in Figure 1. The blood pressure remained at normotensive levels for five hours, thereafter gradually rising to reach its pre-test levels about 12 hours after completion of the infusion.

Upper tracing—brachial artery pressure; lower tracing—EKG, lead V_4; a—Dibenamine started, constant I.V. drip; b—Dibenamine stopped (60 minutes); T—time in minutes from beginning of injection; H.R.—heart rate; B.P.—blood pressure.

was a leukocytosis of 17,000 with a shift to the left on one occasion. During this episode, frequent sedimentation rates were normal. Repeated antistreptolysin titres were reported as less than 50 units. Serial electrocardiograms revealed marked ST-T changes in leads from the left ventricle. The pattern did not permit an exact diagnosis. During this period, the patient had no complaints referable to his heart, except on two occasions when he developed paroxysmal auricular tachycardia which responded readily to carotid sinus pressure. There were no other cardiac abnormalities, and

roentgenograms of the chest showed no change in heart size. He was treated with aspirin and bed rest and the symptoms disappeared in ten days. He was considered to have acute rheumatic fever and operation was deferred.

On February 27, 1950, under endotracheal anesthesia, the abdomen was entered through an inverted "U" incision.* The left kidney and adrenal area was explored first, but nothing unusual, except for a peculiar lobulation of the kidney, was noted. The right kidney was normal and easily mobilized. Above it a rounded mass was found, with a very thin rim of adrenal tissue in its periphery. During manipulation and subsequent removal of the mass, the blood pressure fluctuated markedly and was

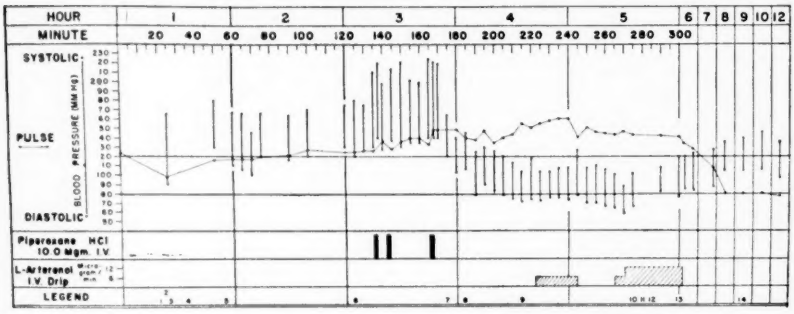

FIG. 3. Case 2. Pheochromocytoma: operation, February 27, 1950.

Legend

1. Pentothal induction (350 mgm.)
2. Cyclopropane—ether begun
3. d—Tubocurarine (6.0 mgm.)
4. Operation begun
5. Explore left adrenal
6. Explore right adrenal
7. Vessels ligated
8. Tumor removed
9. Trendelenburg position
10. Operation finished
11. Eschatin (5.0 cc. I.V.)
12. Lipo-adrenal extract (2.0 cc. I.M.)
13. Returned to room
14. Shock blocks removed

controlled by injections of adrenolytic and pressor drugs† (Fig. 3). The abdomen was carefully explored for additional chromaffin bodies and none was found. Marked arteriosclerotic changes in the abdominal vessels were noted.

The mass measured 7 x 2.5 x 2 cm. and weighed 89 grams. Histological examination established the diagnosis of pheochromocytoma.

A 30-gram sample of the tumor was analyzed.‡ On bioassay it was found to contain 348 mg. of total pressor base, of which arterenol accounted for approximately 58% of the total activity.

The postoperative course was not remarkable and the patient was discharged from

* The operation was performed by Drs. Alfred Hurwitz and Truett Frazier.

† The arterenol used in this study was furnished through the kindness of Winthrop-Stearns, Inc.

‡ We are indebted to Drs. William T. Salter and Desmond D. Bonnycastle, Department of Pharmacology, Yale University School of Medicine, for the analysis. The assays were made utilizing several methods, with good agreement between the results (Auerbach, M. E. and Angell, E.: The determination of arterenol in epinephrine. Science, 1949, *109*, 537; Burn, J. H., Hutcheon, D. E., and Parker, R. H. O.: Estimation of adrenaline-noradrenaline mixtures. Brit. J. Pharm., 1950, *5*, 142).

the hospital on April 20, 1950, markedly improved, although the blood pressure had not returned to normal levels. The electrocardiogram had returned to normal.

On examination four months later, the patient still complained of mild headache. The blood pressure was found to be variable, but frequent normal values were observed during the week that he remained in the hospital. A sedation test was now positive, the blood pressure falling from a control level of 160/110 mm. Hg to 100/84 mm. Hg. There were no marked changes in the cold pressor and postural tests over previous results. The histamine and benzodioxane tests were negative. On the morning that the Dibenamine test was done, control blood pressure readings averaged 130/90 mm. Hg. Following the same dose of Dibenamine as had been given preoperatively, there was no change in the systolic level and a slight rise in the diastolic.

Discussion. This patient again illustrates the importance of considering pheochromocytoma in a young person with hypertension. Whereas the blood pressure was consistently elevated, he was still subject to episodes which were highly suggestive of paroxysmal rises. To be sure, these could not be very well differentiated from anxiety attacks without objective blood pressure measurements, since the patient presented many features considered typical of an anxiety state.

In this instance a diagnosis of pheochromocytoma was made even though the tumor was not localized preoperatively. The metabolic disturbances were consistent with previously reported cases,[2, 16, 18] and the response to histamine, benzodioxane, and Dibenamine provided further confirmation.

The patient's intercurrent illness prior to operation is not entirely explained. It was consistent with rheumatic fever, particularly in view of the history of a similar episode in 1946. Whether either or both of these illnesses are attributable solely to the presence of a tumor with periodic release of humoral substances is not known. The electrocardiographic changes were not entirely characteristic of either pericarditis or myocarditis. They may, perhaps, be explained by myocardial damage produced by large amounts of circulating pressor substances. MacKeith[11] described ST-T changes which persisted for eight weeks following an episode of paroxysmal hypertension and thought they may have been the result of small myocardial hemorrhages such as have been reported in patients dying after paroxysms of hypertension.

Since the introduction of the adrenolytic and sympatholytic drugs, numerous reports of their use as agents in the diagnosis of pheochromocytoma have appeared.[1, 3, 6-9, 11] Grimson and his co-workers[7] were the first to report the use of a short-acting adrenolytic drug (C 7337) during removal of a benign pheochromocytoma. Others have used[1] or recommended[2] the use of the longer-acting Dibenamine in a similar fashion. We favored the use of the shorter-acting benzodioxane during operation because of the danger of the additive effect of Dibenamine during the period of hypotension that usually follows removal of the tumor. Others[7, 10] have expressed a similar opinion.

The effects of epinephrine and arterenol are fairly well understood. It is now known that the pressor response obtained with epinephrine is probably

due to the presence of arterenol in the preparation. Epinephrine itself is predominantly a vasodilator, whereas arterenol produces a marked increase in peripheral resistance.[4, 5, 14] In five normal subjects who were given arterenol, we have noted a significant increase in systolic and diastolic pressures during the infusion.

The effect of arterenol during the operation in this case is difficult to assess owing to its use together with other drugs and additional measures. Indeed it was found that the Trendelenburg position was quite effective in maintaining the blood pressure at adequate levels some time after operation. However, the initial blood pressure rise shortly after starting arterenol and the fall when the drug was discontinued are most suggestive of a pressor effect from the drug.

Although the patient's blood pressure was variable, frequent normal values were obtained on his return to the hospital seven months after operation. Others[1, 15, 18] have reported delayed falls in blood pressure following successful removal of pheochromocytoma. Goldenberg and Aranow[4] suggest that sustained hypertension following removal of a pheochromocytoma may be secondary to the prolonged presence of pressor substances in the blood before operation, and may not require their continued presence for its maintenance. They postulate that this may indicate that neither epinephrine nor arterenol was circulating in excess of the other in such patients. Our finding of an approximately equal proportion of these two amines in this case is consistent with their observations.

Much has been written in regard to the specificity and reliability of the various diagnostic tests for pheochromocytoma. It is now well known that most of the drug tests can yield both false-negative and false-positive results.[1, 2, 8, 14, 16, 18] Furthermore, serious reactions may occur with histamine in patients with pheochromocytoma and with the benzodioxanes in cases of essential hypertension. In patients with sustained hypertension it would appear reasonable, then, to use the histamine test as a screening procedure where there is no evidence of pheochromocytoma. The benzodioxanes, on the other hand, should be used when the presence of pheochromocytoma seems probable.

To illustrate that even this rational approach is not foolproof, it is worthwhile mentioning that we have observed an alarming response to histamine with an unmeasurably high blood pressure and pulmonary edema in a patient who was later proved to have an adrenal cortical adenoma but no pheochromocytoma.

Dibenamine has been used recently in the diagnosis of pheochromocytoma and is thought by some to be specific.[1, 17] Investigation of this drug by Nickerson and Goodman[12] and Hecht and Anderson[10] seems to indicate that Dibenamine will produce a sustained lowering of the blood pressure in those patients with pheochromocytomas which contain large amounts of arterenol. The side effects of Dibenamine can, to a large extent, be controlled by

adjusting the rate of infusion, and we believe that the drug is worthy of further trial. Haimovici and Medinets[9] reported marked blood pressure reduction with the drug in patients with early or moderate essential hypertension, but no significant lowering in patients in the malignant phase. A recent report,[10] however, indicates that Dibenamine will effect significant blood pressure lowering even in the latter group. Our experience with the drug in a small number of hypertensive patients tends to confirm the results of Haimovici and Medinets, but our series is, as yet, too small for adequate evaluation. In five normal subjects, administration of arterenol after premedication with Dibenamine failed to produce the blood pressure elevations that had been noted with arterenol alone, even when the rate of infusion was doubled or tripled.

Pitcairn and Youmans,[13] however, have concluded that Dibenamine will not block uniformly the pressor action of arterenol. It is obvious that more study of the various drugs is necessary before claims as to specificity can be made. At the moment, we feel that Dibenamine cannot be considered specific in pheochromocytoma.

Whereas no valid conclusion regarding the efficacy of arterenol during operation can be drawn, it would appear, on theoretical grounds, at least, that the drug is more useful than epinephrine in preventing peripheral vascular collapse following removal of the pheochromocytoma from a patient who had shown a marked response to Dibenamine preoperatively.

Summary

1. Two cases of pheochromocytoma with assays of the tumors are presented.

2. The rôle of various pharmacologic agents in the symptomatology, diagnosis, and management is discussed.

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ACETYLCHOLINE AND THE CENTRAL NERVOUS SYSTEM

A Review of Neurochemical Mechanisms*

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Since the chemical synthesis of acetylcholine (ACh) was described toward the close of the last century,[53] an extensive literature has developed in regard to its pharmacologic activity, and considerable evidence has been gathered to implicate it in some of the fundamental processes of the nervous system. Information concerning this substance appears to have been gained at first through investigative effort directed toward its general physiological properties as mediated through the autonomic nervous system. As a sequel to this phase in experimental approach, attention was focused next upon its possible rôle in peripheral nerve physiology, and, more recently, rather intense interest has developed as to its place in metabolic processes of the brain. Certain hypotheses have been advanced regarding its participation in the integral physiology of the central nervous system, and some of the manifestations of disturbed physiology as reflected in clinical problems such as epilepsy and head injury, for example, have been attributed to derangement of this aspect of metabolic function. It seems appropriate, therefore, to review the pertinent contributions in this field which serve as foundations upon which current concepts are based.

An active interest in the choline derivatives already existed at the time that Nothnagel (1894) successfully synthesized ACh,[58] by reason of the contemporary pharmacological studies devoted to muscarine and neurine with which there appeared to be close chemical relationship. Mott and Halliburton[47] drew attention to the presence of choline in the cerebrospinal fluid of patients with epileptic manifestations secondary to destructive processes of the brain (general paralysis); however, this remarkable correlation was not entirely appreciated at the time. In the course of their experimental studies, they recognized as well that atropine prevented the fall in blood pressure which ordinarily followed injection of choline, and they thereby provided a groundwork for observations on choline-atropine antagonism. It was during the same period that Reid Hunt[34] in 1901 isolated a blood pressure lowering principle in aqueous extract from suprarenal tissue, sympathetic ganglia, and brain, and suggested that this substance might be an "ester-like body containing choline in its molecule." In a later communication, Hunt and Taveau[35] discussed the marked potency of the acetylated form compared with choline and its other derivatives in depressing blood pressure and respiration. Shortly thereafter, Frank and

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Isaac[24] in 1909 and Meyer[41] in 1912 proposed general pharmacological parallelisms between (i) choline derivatives and the parasympathetic nervous system, and (ii) adrenalin and the sympathetic nervous system.

However, formulation of a sound basis for pharmacological study of the autonomic nervous system may be properly attributed to Langley,[40] who extended and developed the pioneer efforts of Gaskell[20] in the physiology of the "involuntary" nervous system. An appreciation of the neurochemical antagonisms existing within this system is set down in Langley's monograph, *Autonomic nervous system*. Much of the available information concerning the activity of the parasympathetic component up to this time, however, had been derived from observations on the effects induced by pilocarpine.

Largely through the encouragement of Dale, the chemical identification of ACh as an active principle of ergot was established by Ewins[18, 19] in 1914. The comparative biological actions of the choline derivatives, esters, ethers, and ACh were investigated extensively by Dale[14] who reported on their properties in detail.

ACh and electrocortical activity

Some historically important observations concerning the action of parasympathetic drugs have been made by employing the intraventricular route of administration. While studying the intraventricular effects of pituitrin, Cushing[13] noted that its action strikingly resembled that of pilocarpine. When pilocarpine was injected intraventricularly, the effects were much greater than those seen when the subcutaneous route was employed; body temperature fell appreciably, and sweating, salivation, retching, and vomiting were extremely severe at times. The mechanism was believed to involve central autonomic stimulation, being predominantly parasympathetic in character. Atropinization by the subcutaneous route or by intraventricular instillation counteracted these effects.

Intraventricular injection of ACh in unanesthetized cats[15, 16] resulted almost invariably in temporary cessation of respiration followed by a sleep-like state lasting several hours. Irregularities of the heart, similar to those seen after stimulation of the central end of the vagus nerve, were observed as well.[16] The intraventricular route was also employed in clinical studies, where onset of nausea, sweating, and often vomiting were observed.[38] Other early observations on the central nervous system effects of ACh were made using the *encéphale isolé* preparation in cats.[4, 6] In these instances, carotid injection of ACh produced increased frequency of the "Berger waves" emanating from the cortex, and electroencephalographic patterns resembling those which accompany the awakening reaction were noted.

With the development of electroencephalographic techniques, studies concerning the neuropharmacological action of acetylcholine were extended to observations on the cerebral cortex. Sjöstrand[50] found that changes in

electrical potentials induced by strychninization of rabbit cortex were enhanced if ACh or eserine was applied before, simultaneously with, or subsequent to strychninization. Miller and his co-workers[46] noted that the topical application of ACh to eserinized cortex was attended by spike discharges in the electrocorticogram and occasionally by motor responses; atropinization of these animals prevented both the electrographic and motor phenomena.

Chatfield and Dempsey[12] observed characteristic changes in the electrocorticogram of cats when various areas of eserinized cortex (somesthetic, auditory, and motor cortex) were treated with 1 per cent ACh. These changes consisted of increased spontaneous electrocortical activity, the appearance of 5 to 10 per second spike discharges and, later 20 to 30 per second low amplitude spikes which spread to certain other cortical areas. Atropinization was found to abolish interval spike potentials but not the ACh bursts per se. Brenner and Merritt[7] found, however, that topical ACh in concentrations of 2.5 to 10 per cent was followed by spike discharges without resort to eserine, and that the electrographic manifestations were not inhibited by atropine, 1 mg. per kg. They suggested that the effect of ACh and other choline derivatives upon the cerebral cortex is comparable to the action of ACh upon sympathetic ganglia and at the skeletal myoneural junction. Forster[23] established the relationship of focal motor phenomena of the seizure discharge with ACh-induced spike discharges in the electrocorticogram.

Kristiansen and Courtois,[39] by demonstrating that isolated cerebral cortex responds with rhythmic spike discharges after topical application of ACh, have excluded thalamo-cortical connections as essential for the synchronization of neuronal activity which results in spike discharges. Beckett and Gellhorn[2] observed that when ACh was applied to "suppressor areas" of cat and macaque cortex, local suppression of electrocortical activity occurred and likewise loss of spontaneous or electrically induced movements. They found that ACh augmented action potentials evoked by peripheral stimulation and recorded from somesthetic receiving areas; cholinesterase (ChE) applied topically to cortex had the opposite effect. In a later study, Hyde, Beckett, and Gellhorn[36] reported that the effect of eserine, whether given intravenously or applied topically to the cerebral cortex, potentiated the activity of a wide group of chemically different convulsant agents and they suggested that ACh might be employed for activation of the electroencephalogram. However, Kaufman, Marshall, and Walker,[38] in clinical studies on activation of the electroencephalogram (EEG), failed to find any significant alteration in the pattern of the EEG after intravenous administration of ACh.

Jacksonian seizures have been produced in unanesthetized rabbits by topical application of ACh to the masticatory cortex. Eserinization of the cortex enhanced the convulsive manifestations, whereas intravenous atro-

pine abolished these seizure discharges.[1] Marrazzi and Hart[42] observed that when ACh is injected into the carotid artery, potentiation of the response of the visual cortex to optic nerve stimulation occurs. Di-isopropyl-fluorophosphate (DFP) also enhanced these responses while atropine diminished them.[43]

Cholinergic areas of the central nervous system

Methods of determining choline acetylase (ChA) activity have made possible the investigation of the propensity of different regions of the central nervous system to synthesize ACh.[51] Based upon this method of assay, nuclear masses and tracts of the nervous system have been classified as belonging predominantly to either cholinergic or non-cholinergic systems.[22] The topographically distinct areas which display high levels of ChA activity (cholinergic group) include the anterior horn cells, anterior roots, and the motor nuclei of the cranial nerves. The pyramidal tracts uniformly show low ChA activity. The motor cortex appears to have an intermediate level of ChA activity, the inference being that cholinergic fibers converge upon the pyramidal cell population which is per se non-cholinergic. The fact that topical application of eserine and ACh to motor cortex causes discharge of impulses in the pyramidal tracts would appear to strengthen the view that cholinergic fibers may fire the non-cholinergic neurones of the pyramidal system.

This general pattern of organization, namely, a succession of alternating cholinergic and non-cholinergic neuronal aggregates, has also been demonstrated by ChA determinations using the ascending sensory pathways of the spinal cord. The posterior roots and the tracts of the dorsal columns of the cord have been shown to be non-cholinergic, while the nuclei gracilis and cuneatus are cholinergic. Generally, the second order ascending fibers (lateral lemniscus and spinocerebellar tracts) show intermediate values of ChA activity, whereas the third order neurone of the afferent path seems to be non-cholinergic. The supraoptic nucleus possesses high ChA activity. This finding is consistent with its ease of excitation by ACh or DFP.[17] Similarly, the caudate nucleus, red nucleus, and hippocampal formation show moderately high ChA values. An alternating system of cholinergic and non-cholinergic neurones has been shown to exist in the cerebellum and its projections as well as within relay pathways of the visual system.

Species variations in ChA activity suggests that an element of caution be taken in the interpretation and acceptance of strict categorization of different chemical neuronal systems within the central nervous system. Nevertheless, additional indirect evidence for the presence of cholinergic and non-cholinergic neuronal systems within the cerebral cortex seems to be the selective inhibition of ACh-induced cortical spike discharges, by intravenous atropine sulphate and adenine nucleotides in doses which are completely ineffective in diminishing strychnine-induced spikes or in altering the level of spontaneous electrocortical activity (see below).[58]

ACh-atropine antagonism within the central nervous system

The pharmacological antagonism of atropine to the muscarinic effects of ACh is well known.[14] Atropinization of an animal will prevent the development of spike potentials which usually follows the topical application of ACh to cerebral cortex.[40] Intraventricular injection of atropine in conjunction with ACh does not result in spike discharges usually seen when ACh is given alone.[33] However, others have found that atropinization (1 mg./kg.) failed to counteract the effects of ACh applied topically or injected into the subarachnoid cisterns, and they have concluded that its action upon cerebral cortex more closely resembled that upon sympathetic ganglia and parasympathetic end organs.[7] Other experimental observations indicate that this atropinizing dose is relatively ineffecive for ACh antagonism and that greater doses (2-5 mg./kg.) are necessary for the inhibition of ACh-induced spike discharges without necessarily causing depression of spontaneous electrocortical activity.[58]

The clinical studies of Williams[97] demonstrate that atropine inhibits the frequency or ease of induction of petit mal attacks precipitated by the injection of eserine or ACh in patients with petit mal epilepsy. Another interesting phenomenon, although by no means completely understood, is the clinical and subjective improvement which occurs in the Parkinsonian syndrome following the use of atropine or related compounds. The site of the pathological process in this disorder is usually located in the basal ganglia which have been shown to be predominantly cholinergic.[29] It seems reasonable to speculate on the possibility of a derangement of ACh metabolism as the underlying mechanism responsible for the clinical manifestations attending this syndrome. The amelioration which occurs with atropine may be related to ACh antagonism at this or other neural levels.

In recent years, studies of metabolic changes in cerebral tissue following experimental head injuries have disclosed significant alterations of fundamental neurochemical mechanisms.[85] Traumatized areas of cortex show appreciable increases in lactic acid and inorganic phosphate content, whereas phosphocreatine and ATP levels are decreased. The adenine nucleotides, of which ATP is the most potent member, comprise the "adenylic acid system" which serves as the chief regulatory mechanism of oxidative glucose metabolism. Bornstein[5] has clearly demonstrated that free ACh, which is not present normally in the cerebrospinal fluid (CSF), appears in significant amounts following experimental cerebral trauma.

There appears to be a strong correlation between the amount of ACh which appears in the CSF, and behavioral and EEG changes following experimental brain concussion. That the elaboration of free ACh following cerebral trauma is responsible for the acute paralytic state and the later excitatory phenomena may be inferred from these experimental data. Bornstein[5] found that atropine abolished the stupor and abnormal EEG patterns seen as sequelae of experimental brain trauma. Atropine relieved

behavioral and EEG changes produced by intracisternal injection of ACh. These alterations closely simulated the manifestations following experimental head injuries described above. Tower and McEachern observed low ChE activity and liberation of free ACh in the CSF of patients with head injuries[63] and also in epileptics.[64] With clinical recovery, these indices of chemical change reverted to normal. Further, there seemed to be close correlation between ChE activity, ACh level of the CSF, degree of EEG abnormality, and the clinical state of the patient. Ward[65] employed atropine in the treatment of patients with severe closed head injuries having appreciable cerebral damage as evidenced by depth of coma, focal neurological signs, and grossly bloody CSF. The rationale of treatment was based on the aforementioned experimental evidence[5, 62, 63] that the chemical changes associated with anatomical damage can be vitiated by anticholinergic agents. Definite improvement was seen in the state of consciousness in the patients so treated.

Anticholinesterases

The anticholinesterases may produce states of increased electrocortical excitability comparable in many ways to those seen following topical application of ACh.[29] Although it has been generally assumed that the mechanism of action is mediated through the accumulation of undestroyed ACh, this is by no means certain.[21] However, whether the anticholinesterases excite neuronal elements directly by reason of their chemical structure, or cause inhibition of ChE by some chemical union with this substance, is still a matter of speculation. The latter mechanism probably is involved in the action of eserine, for, when this substance is applied directly to cerebral cortex, rarely is the electrocortical pattern so affected as to result in spike discharges. Similar to eserine are the mechanisms of action of DFP and tetra-ethylprophosphate (TEP);[21] however, their combination with ChE appears to be relatively irreversible.[50] These agents also affect cell respiratory functions of brain tissue, and may possibly exert their effects secondarily through a disturbance in cerebral oxidative metabolism.[8, 45]

Propagation of the nerve impulse in isolated nerve preparations requires the integrity of certain metabolic processes; e.g., the phosphorylation cycle, the synthesis and breakdown of ACh by ChA and ChE. The experimental findings of Nachmansohn and Feld[50] indicate that DFP acts relatively slowly but progressively in inhibiting ChE, which makes determinations of ChE activity over regular intervals possible. Exposure of the stellar or proximal fin nerve on the giant axon of the squid to 0.01 molar solution of eserine has been shown to abolish the action potential, but this effect is reversible inasmuch as the state of excitability may be restored by bathing the nerve preparation in sea water.[10] However, under similar experimental conditions, DFP abolished conduction of the nerve impulse when the ChE was reduced to 3 per cent or less of the control level.[9] These

observations show further the integral rôle of ACh and ChE in the mechanism of nerve conduction. The excitability of cortical neurones is diminished by ChE and augumented by eserine; topical application of eserine and ACh to cortical suppressor areas, however, may produce a local suppression of spontaneous electrocortical activity.[2]

EEG changes similar to those seen clinically in status epilepticus have been observed following injection of DFP into the common carotid artery of rabbits. These changes were associated with markedly diminished ChE activity of the cortex.[25, 80] The normal electrocortical pattern could be re-established in these animals by atropinization, The level of ChE following the administration of DFP appears to be inversely proportional to the varying degrees of abnormality in the electrocorticogram. According to Nachmansohn and Feld,[50] abnormal EEG patterns were produced only after an appreciable decrease in the level of ChE activity occurred and, therefore, there seems to be a wide safety margin in the maintenance of normal neuronal excitability. Complete inactivation of ChE, however, is apparently incompatible with life.

The injection of DFP into the supraoptic nucleus results in a decrease in urine flow in dogs.[17] The mechanism is presumably one of prolonged stimulation of this nucleus by the accumulation of ACh, which results in an increased liberation of antidiuretic hormone.

DFP has received extensive investigation in clinical medicine.[28, 29, 54] This agent has been employed in the treatment of abdominal distention in conjunction with neostigmine and appears to potentiate the effect of neostigmine in relieving this condition. However, patients who received this treatment experienced untoward symptoms consisting of excessive dreaming, nightmares, paresthesias and delirium, which were found to be diminished largely by the administration of atropine. Clinical investigations have revealed that DFP causes an increase in electrocortical activity, increases in the predominant cerebral rhythms, paroxysmal irregularities of rhythm, and occasionally high voltage slow activity most marked in frontal leads. Atropine was observed to have a beneficial effect in approximately 50 per cent of their patients who showed abnormal EEG findings after administration of DFP.[29]

Although the mechanism through which DFP exerts its effects upon the central nervous system is not entirely clear, the indirect evidence of atropine antagonism suggests that perhaps its action is intimately related to altered ACh metabolism.

The rôle of the adenine nucleotides in central nervous system metabolism

It has been shown that ATP is necessary for the *in vitro* synthesis of ACh, and that the energy derived from the splitting of the organic phosphate linkages of ATP is apparently utilized in this synthesis.[51] The observations of Stone, *et al.*[62] on chemical changes following experimental

head injuries disclosed that areas of contused brain have increased lactic acid and inorganic phosphate levels, while a decrease in phosphocreatine and ATP content consistently occurs. The relationship of adenine nucleotides (adenylic acid system) to the convulsant action of ACh has come under study recently.[37, 56] ATP and other adenine derivatives applied topically or injected intravenously inhibit characteristic ACh-induced high voltage electrocortical discharges.[37, 56] These findings suggest that adenine derivatives may have a place in the treatment of certain clinical disorders, i.e., seizure disturbances and head injuries.[57] The mechanism of this inhibition is not known. However, it is possible that excessive concentration of ACh may cause depletion or retard formation of ATP, allowing the neuronal pool to respond to ACh by synchronous discharge. Increasing the ATP concentration either by topical application or intravenous administration may then restore the chemical equilibrium. There is at present, however, no proof for this proposed mechanism.

The ACh content of brain has been shown to vary with its physiological state; it is elevated during sleep and anesthesia, whereas decreased levels occur during emotional excitement, convulsions, and following electrical stimulation of the brain.[55] The destruction and resynthesis of ACh *in vivo* have been found to be rapidly reversible. Attention has been directed also to the findings that during anesthesia (when ACh synthesis would be expected to be minimal), brain lactic acid level is low and the phosphocreatine level is high. During electrical stimulation or convulsions the relationships between lactic acid and phosphocreatine are reversed, possibly indicating an increase in ACh synthesis. It was felt that the increased frequency of seizures and EEG changes seen clinically in epileptic patients during the early stages of anesthesia and sleep may be due to high ACh levels in the brain resulting from minimal utilization of ACh during these states. Although the neuronal excitability under these conditions is decreased, ACh may accumulate to a level which will stimulate and discharge neuronal aggregates, whereas deeper anesthesia may interfere with metabolic processes generally and thereby prevent excessive accumulation of ACh.

The inferences gained from observations on nucleotide and ACh levels, and their correlation with physiological activity of the cortex, aid in some measure in evaluating the rôle of ACh in the central nervous system.

Other mediators in the central nervous system

Until recent years, the rôle of ACh has been preëminent in discussions of the chemical theory of conduction and synaptic transmission. Then, in 1948, Hellauer and Umrath[31, 32] postulated another as yet unidentified agent as being involved in synaptic transmission and as producing vasodilatation, including that seen on antidromic stimulation. They concluded that this "ferment" is subject to enzymatic destruction and that strychnine inhibits this process. Strychnine-induced electrocortical discharges are

dissimilar to those caused by ACh: the latter produces bursts of high amplitude negative spikes occurring usually at a frequency of about 10-15 per second. Strychnine spikes are of somewhat higher amplitude, customarily accompanied by an initial positive deflection, and recur singularly usually every 5 to 10 seconds, although the frequency of firing is variable. The underlying mechanism of this firing process would seem to be different, since the action of atropine and adenine nucleotides on spike discharges induced by these two agents is quite dissimilar.

Observations on the effect of ACh on the cerebellar cortex have disclosed that ACh is relatively ineffective in inducing spike discharges.[58] Since high amplitude spike discharges are presumably the result of synchronous firing of neuronal groups, the closely connected cells of the cerebellar cortex would seem to provide an ideal location for such synchrony to occur. The inefficacy of ACh applied to the cerebellar cortex would seem to refute either the theory of spike production or the theory of universality of ACh transmission; but other findings such as the variability of effect of ACh on different areas of cortex seem to support the view that there may be more than one chemical mediator responsible for synaptic transmission. Mention might also be made of the variable clinical and EEG patterns observed in cryptogenic epilepsy, and of the relative specificity of drugs used in their treatment—tridione in petit mal, phenurone in psychomotor seizures, and dilantin and phenobarbital in grand mal. This near therapeutic specificity may be indicative of derangements in different chemical mechanisms involved in synaptic transmission.

Chemical theory of conduction and transmission

In 1945, Feldberg[30] in assessing the rôle of ACh in the CNS, outlined its status as a "central synaptic transmittor" of nerve impulses. He stated that the "presence of ACh and ChE in central nervous tissue, the ability of such tissue to synthesize ACh and to release it under certain conditions, the central effects of ACh and of eserine, all provide strong evidence in favor of the theory that the transmission across a number of synapses in the central pathway of autonomic and motor neurones occurs through the mediation of ACh." Some of the objections to the acceptance of this thesis which regards ACh as the universal central transmittor were also considered. In the ascending phylogenetic scale the more developed brains have appeared to become less dependent upon ACh metabolism in that the ability to synthesize ACh, the concentration of ChE, and the ACh content of the nervous tissue are all decreased. The finding of cholinergic and noncholinergic neurones and tracts, and a low ACh content in cerebellar cortex which is rich in synaptic relationships, throws some doubt concerning the universality of ACh in central transmission and conduction. However, important evidence against the alternative theory which considers the action current as the synaptic transmittor was cited by Feldberg,[30] as follows:

the inability of the impulse to cross the synapse in sympathetic ganglia when the ACh content of the nerve had been markedly reduced and the ability of the nerve to synthesize ACh had been lost, and the observation that only cholinergic fibers are able to make functional connection with sympathetic ganglia in regeneration experiments.[43]

Nachmansohn in 1946,[49] in regarding ACh as essential in conduction and transmission, based his theory upon the following evidence. From observations on the electric eel, the concentration of ChE in nerve tissue was found to be high enough to permit an enzymatic process whose time relations are commensurate with electric manifestations of nerve impulses.[48, 50] The localization of ChE at those neuronal surfaces which present bioelectrical phenomena contrasts remarkably with the localization of other enzymes in the body.[3] The formation of ACh by ChA has been found to occur at a rapid rate. A direct proportionality between ChE activity and the voltage of current exists in the electric organ of *Electophorus electricus*,[52] and it is generally agreed that the high energy bonds of ATP provide the energy necessary for ACh synthesis.[49] Finally, the ability of anticholinesterases to diminish and, in high concentrations, to abolish the action potential in nerve[9, 10] has been shown, and this adds to the necessity of an intact ACh metabolic process for impulse conduction. Whitteridge[98] feels that the action of ACh in the central nervous system is mainly concerned with the "facilitation of repetitive activity, particularly in the interneurones," but he does not elaborate further on the method of production of this facilitation.

Conclusions

The neurochemical studies of Feldberg and Vogt[22] have shown that there exists within the central nervous system a pattern of chemical neuronal organization consisting of alternating cholinergic and non-cholinergic neurone pools. This relatively new concept may possibly clarify some observations upon chemically induced electrocortical activity. It has been shown that synchronous electrocortical discharges which occur following strychninization of cerebral cortex are unaffected by atropine or adenine compounds, whereas these agents inhibit cortical spike potentials caused by ACh.[56] These observations may be partially explained on the basis of Feldberg's thesis concerning cholinergic and non-cholinergic neurones, in that the chemical mechanisms involved in inducing synchronous firing following strychninization may include chemical synaptic mediators other than ACh. Appropriate chemical agents may be necessary for the initiation of neuronal excitation and discharge depending upon whether the neuronal pool activated belongs predominantly to cholinergic or non-cholinergic groups. The ineffectiveness of the adenine nucleotides and atropine in depressing strychnine-induced electrocortical activity, on the other hand, may point to some other mechanism of strychnine action as suggested by

Chang, who believes that this agent decreases the latency of synaptic transmission and increases the chance for synchronous neuronal firing.[11] It has also been suggested that strychnine increases oxidative processes in cell metabolism.[61]

Recent contributions from the field of clinical medicine point to the importance of ACh metabolism in cerebral function. The genesis of cryptogenic epilepsy has been linked to disturbances in ACh metabolism, while correlations between ChE activity and EEG pattern seem to support this hypothesis.[64] ACh has been used to precipitate clinical seizures, whereas atropine has been shown to afford protection against seizures induced by such methods. The appearance of ACh in the CSF in appreciable amounts following severe head injury may be a secondary manifestation of the altered nucleotide metabolism which has been shown to accompany trauma to brain tissue.

The arguments upon which the mechanisms of initiation and propagation of the nerve impulse are based are somewhat weighted toward the consideration that these processes are predominantly if not wholly electrical in nature. Gerard[27] has pointed out many discrepancies in the arguments pointing to importance of ACh, but feels that the long duration of some synaptic potentials can only be explained on the basis of some chemical reaction which, however, may be entirely unrelated to ACh metabolism. Since energy transfer is involved in the production of the electrical manifestations of synaptic transmission and nerve conduction, it seems logical to assume that this energy must be derived from chemical reactions. It is the purpose of this review to focus attention on those investigations which have sought to uncover some of the fundamental neurochemical processes involved in central neural activity.

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THE INTERRELATIONSHIPS OF FOODSTUFFS IN METABOLISM

A Tentative Proposal*

JOHN P. PETERS

Like every panel of physiology or medicine, metabolism has been studied and taught by the categorical method. But, for a full understanding of the subject, synthesis and integration are essential. Energy production and caloric needs are only significant when the purposes to which the energy is directed are taken into account. It may be useful to regard the body as a complex industrial plant or machine. Power to run this plant could be derived from a variety of fuels, such as wood, coal, or oil. The counterparts of these in the physiological factory are carbohydrate, fat, and protein. The mere combustion of these for the production of power may be called the energy metabolism. The efficiency of the power plant will vary—first, with the nature of the fuel; secondly, with the device used for its combustion. This will have to be different and appropriate for each fuel. The mere production of heat, though essential for its operation, is not, however, the object of this plant. The heat must be directed to useful ends. This requires highly specialized machinery for which particular materials must be provided. Some of these can be manufactured on the premises; others must be supplied ready-made. This machinery must be integrated and must be equipped with regulating devices to permit variations in the relative speeds of its multifarious processes. It has been suggested that these phases of metabolism be termed *operative* metabolism, in contradistinction to *energy* metabolism. Until recently operative metabolism has been an almost unpenetrated mystery. This mystery is now being dispelled with such rapidity that it is hard to keep pace with the advances. It is impossible in a single article to detail these discoveries; an attempt will be made through certain examples to convey some impression of the modern dynamic concept of metabolism which deals with chemical compounds not as mere substances, but as instruments.

Since provision of power is the ultimate limiting factor, it may as well receive attention first. Any one of the three foodstuffs—fat, carbohydrate, or protein—may serve as fuel for this plant, but not indifferently. No one of them is altogether indispensible because each has an operative as well as an energy-producing function. A small amount of carbohydrate is essential for purposes that will be outlined later. If adequate and continuous amounts are not supplied in the food, the body is compelled to manufacture it. The only

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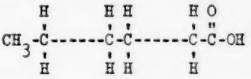
material suitable for this purpose is protein; but to use protein for this purpose is inefficient and wasteful. Species vary in the quantities of carbohydrate required for operative purposes, carnivores being particularly economical in this respect. In the investigation of physiological problems the choice of animals is not a matter of indifference. Inferences with respect to the functions of man cannot be indiscriminately drawn from experiments on rats, rabbits, dogs, hamsters, or even monkeys. Conceptions and misconceptions about diabetes might be very different if von Mehring and Minkowski had chosen another animal than the dog for their classical experiments.

Fat, like carbohydrate, is composed solely of carbon, hydrogen, and oxygen. It is an ideal fuel because its caloric value per unit of mass is so great. It is formed freely in the body from carbohydrate; but, for reasons that will be discussed later, the reverse is not true. The reaction,

1] Carbohydrate ⟶ Fat

is essentially irreversible. Fat would appear to be an indifferent substance which would not have to be provided from the environment. So far as its fuel value is concerned, this is true. Nevertheless, if an animal is given a diet devoid of fat, but containing enough carbohydrate to supply its caloric needs, it will lose weight and ultimately die with characteristic lesions, including a fatty liver and degeneration of the kidneys. The explanation for this lies in the fact that certain types of fatty acids essential for life cannot be formed in the body. A fatty acid consists of a long straight hydrocarbon chain with a carboxyl tail,

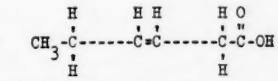
2] Fatty acid, saturated



Stearic acid contains 18 carbons

The length of the carbon chain varies up to about 22 carbons. Of these, fatty acids up to 18 carbons, stearic acid, can be formed in the body. Of the fatty acids in the body, a certain number are unsaturated; that is, they contain carbon-linkages without the full complement of hydrogens.

3] Fatty acid, unsaturated



Oleic acid contains 18 carbons with one unsaturated bond

Oleic acid belongs to this class, being an 18-carbon acid with one unsaturated bond. The body is able to manufacture, from carbohydrate or from fatty acids, oleic acid and other fatty acids with one unsaturated bond. There are, however, in the body certain essential fatty acids containing 18 or more carbons and having more than one unsaturated bond, the principal

ones being linoleic, linolenic, and arachidonic, which cannot be synthesized in the body, but must be provided in fats in the food.

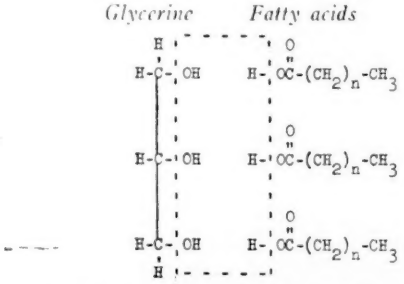
Essential fatty acids which cannot be synthesized in the body:

4]

Linoleic acid	18 carbons, 2 unsaturated bonds
Linolenic acid	18 carbons, 3 unsaturated bonds
Arachidonic acid	20 carbons, 4 unsaturated bonds

A fat is composed of three molecules of fatty acid combined with a molecule of glycerine.

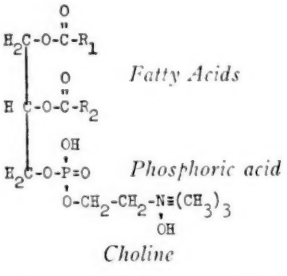
5]



The OH^- and H^+ ions inclosed by the broken lines are lost as water when glycerine and fatty acids combine to form fat.

Fats are found in adipose tissue and are both found in and utilized by the cells of the liver. They do not occur in other tissue cells, however, although fatty acids are burned by these cells. In the tissue cells fatty acids occur in cholesterol esters and phospholipids. The tissue cells apparently burn the fatty acids of the phospholipids, which presumably serve as vehicles for the fatty acids and as instruments that facilitate their oxidation. A characteristic phospholipid, lecithin, has the general composition of a fat, except that one of the fatty acids is replaced by a phosphoric acid and the methylated nitrogen-containing compound, choline.

6]



Of the two fatty acids, R_1 and R_2, one is of the ordinary type, the other belongs to the highly unsaturated types. It is the ordinary saturated fatty acid that is used for fuel, the unsaturated members are conserved with great

economy. They form essential parts of the machinery. It is apparently for the formation of these components of the phospholipids that the essential fatty acids are required.

Carbohydrate and fat are both composed solely of the three elements, carbon, hydrogen, and oxygen, and can, therefore, be completely oxidized to carbon dioxide and water. Protein, on the other hand, contains nitrogen. On this account it cannot be replaced by either carbohydrate or fat. On the other hand, it can, to a certain extent, replace both the other foodstuffs. It is not, however, an efficient fuel because it cannot be completely oxidized. Furthermore, it serves operative functions of such importance that it is not economical to waste it for fuel alone. Proteins are complex combinations of amino acids which have the following general configuration:

7] The radical R differentiates the amino acids.

Although the amino acids have this common characteristic, they differ widely in the nature of the radical, R. Altogether 23 amino acids are generally recognized. Of these 10 cannot be synthesized in the body, but must be provided in the diet. These are generally termed essential amino acids, not because they are any more essential than the others, but because, if all of them are not provided in sufficient quantities and proper proportions, life cannot be supported. It is, therefore, not only necessary to supply adequate amounts of protein in the diet, but also protein of a satisfactory quality. Furthermore, if protein is consumed as fuel, larger amounts are required. The amino acids generally recognized are listed below with the ten essential ones capitalized:

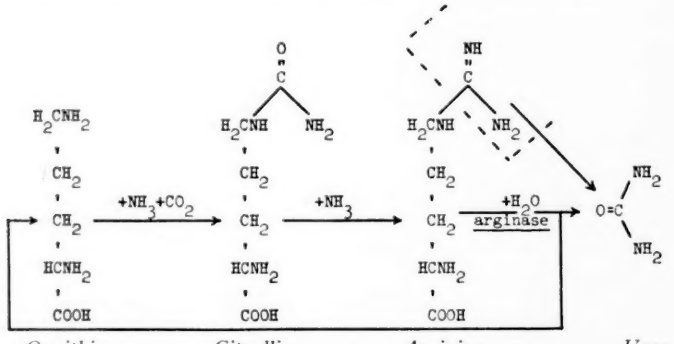
8]

ARGININE	Serine
Citrulline	THREONINE
LYSINE	VALINE
Hydroxylysine	LEUCINE
HISTIDINE	ISOLEUCINE
PHENYLALANINE	Norleucine
Tyrosine	METHIONINE
TRYPTOPHANE	Cystine
Proline	Glutamic acid
Hydroxyproline	Hydroxyglutamic acid
Glycine	Aspartic acid
Alanine	

The precise functions which all of these serve are not known, although most of them occur in varying proportions and in innumerable permutations and combinations in proteins. It may be worth while to point out a few of the processes in which some of them are known to participate. The position of arginine among the ten essential amino acids is open to question. It can apparently be synthesized in the body, but only to a limited extent. It is the

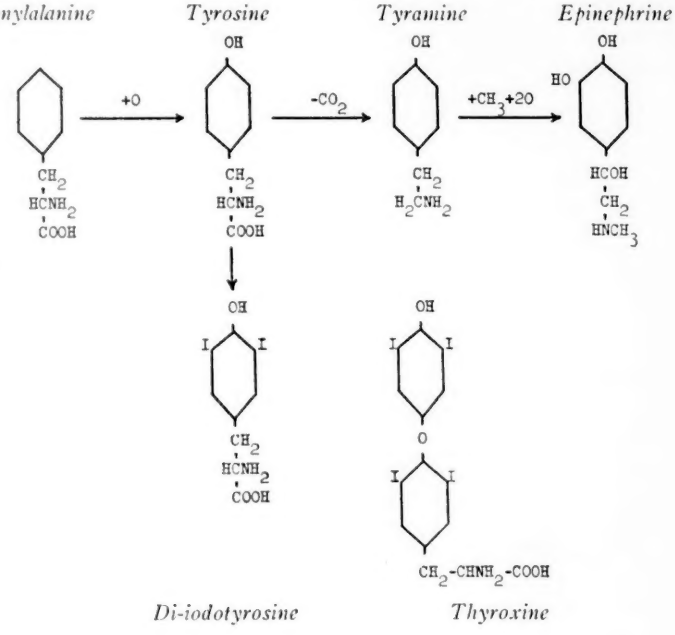
chief vehicle by which ammonia is conveyed to the liver for the formation of urea. The reactions involved in this process are illustrated thus:

9]



Ornithine, an amino acid, for some reason not included in the regular lists, combines with carbon dioxide and ammonia to form citrulline, which is listed: This, in turn, combines with another molecule of ammonia to form arginine plus a molecule of water. From arginine with the addition of a molecule of water and the action of the enzyme, arginase, the amidine group

10]

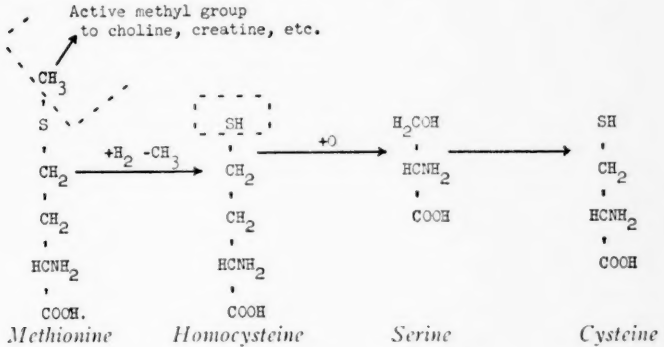


is broken off to form urea and by this reaction ornithine is again reconstituted to repeat the cycle. Arginine also contributes the amidine group for the formation of creatine (see 11]).

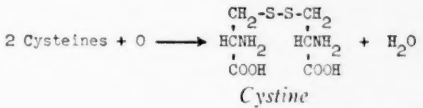
Phenylalanine can be used to form tyrosine, if this is not included in the diet (See 10]).

The former must have also certain independent functions that have not been clearly elucidated, because no amount of tyrosine can be substituted for phenylalanine. The oxidation of phenylalanine to tyrosine is, therefore, an irreversible process. From the latter both epinephrine and thyroxine appear to be elaborated.

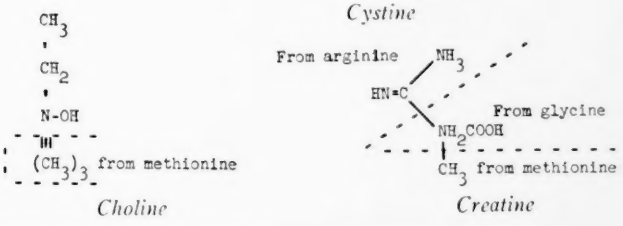
Methionine is peculiar in that it contributes fractions of itself for a variety of purposes.



11] *Methionine* *Homocysteine* *Serine* *Cysteine*



Cystine



Choline *Creatine*

The methyl group, —CH_3, at the top provides active methyl groups for both of the essential substances, choline and creatine. The sulfur group is used for the formation of cystine, cysteine, and secondarily glutathione and other sulfhydryl compounds, with the aid of another amino acid, serine, which can be synthesized in the body.

In addition to the replacement of protein in the body, therefore, proteins must supply amino acids for all kinds of specific purposes. This entails the continuous degradation of a certain amount of protein. The capacity to store protein is extremely limited in a well-fed individual. In spite of its high value, therefore, any excess of protein is burned. In the process of combustion, the nitrogen is broken off as ammonia which is excreted as such, or as urea; the remainder is converted to materials that can be oxidized through the channels of either carbohydrate or fat.

12] Energy-production → Urea or ammonia
Energy-production → Carbohydrate or fatty acid → $CO_2 + H_2O$
Operative → Creatine, creatinine, uric acid, etc.
Operative → Carbohydrate

The end-products of the combustion of protein are, therefore, CO_2, H_2O, and urea (for our purposes the small amounts of sulfate can be neglected). In the urine, in addition to urea and ammonia, other less completely catabolized nitrogen-containing compounds, such as creatinine, uric acid, and amino acids are found. When the protein in the diet is varied, the excretion of urea + ammonia varies accordingly; but the excretion of other nitrogenous substances remains almost entirely unchanged. If protein catabolism is reduced to a minimum by administration of a protein-free high calorie diet, urine urea+ammonia diminishes almost to the vanishing point, but creatinine and uric acid are hardly affected at all, even when energy-production is greatly increased by exercise. This is, perhaps, the best illustration of the distinction between energy-producing and operating metabolism. Creatinine and uric acid are products of operational functions (what has been called endogenous metabolism) which must continue uninterrupted to maintain vital activities. There are no substitutes for the compounds used in these operations. Urea and ammonia are products of energy-producing processes. They, therefore, vary with the nature and quantity of food available. In addition, even the energy-producing function of protein is not entirely independent of operative needs. Protein is the only source of carbohydrate when the latter is not provided in the food and must, under these circumstances, take over the operating offices of carbohydrate.

The proteins themselves must not be regarded as mere structural components of the tissues, but as extremely active agents. Perhaps more than any others they are responsible for the differentiation and specialization of vital activities. Schoenheimer showed that their structures are continually changing. Among other things, proteins form the cores of all enzymes.

There is another large and heterogeneous class of substances for the supply of which animals are dependent upon the environment—the vitamins. There is space for only a few remarks about these compounds. It is unfortunate that such a heterogeneous aggregation of substances should continue to be grouped together under a term that was devised as a cloak

for ignorance, and should be distinguished from one another only by letters and numbers. The composition of a large proportion of these substances is now known; some of their functions have been elucidated. They are no longer mysterious vital forces, but identified chemical compounds that participate in the reactions that constitute the operative metabolism. The majority, especially of the members of the B-vitamin group, are components of enzyme systems.

The nature of these enzyme systems requires a few words. Some of the mysteries surrounding these are also being gradually dispelled. They have also proved to be complexes which can be isolated and identified and which participate in the chemical reactions which they facilitate. The term, enzyme systems, is used because such an organism as the yeast cell forms alcohol from sugar by a long series of linked reactions, each one of which is implemented by a separate enzyme. In most instances an enzyme consists of a highly specialized protein, together with one or more organic components (this is the rôle of the vitamins) and frequently inorganic elements, especially phosphorus. These complexes implement chemical reactions. For example, inorganic phosphate mixed with glycogen is altogether inert.

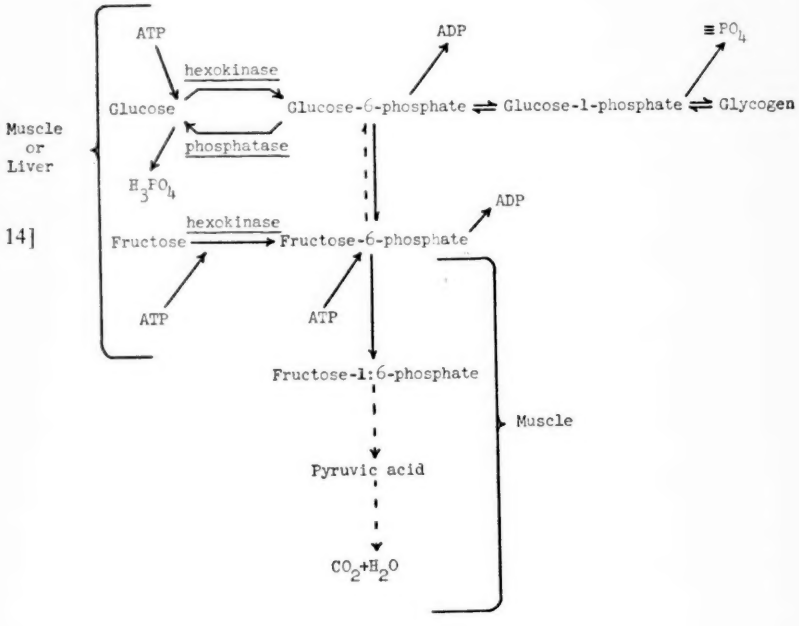
$$\text{Glycogen} + H_3PO_4 = \text{glycogen} + H_3PO_4 \text{ (no enzyme present)}$$

13]

$$\text{Glycogen} + H_3PO_4 \xrightleftharpoons{\text{phosphorylase}} \text{glucose-1-phosphate}$$

But, if the enzyme, phosphorylase, is added to the mixture with a little adenylic acid, glucose-1-phosphate is formed. This reaction is depicted as freely reversible, which it is when this particular set of reagents is allowed to react alone. Under these circumstances, also, the enzyme tends to deteriorate. In its native habitat, however, the reaction is oriented, proceeding predominantly in one direction, because it is linked with other similar reactions. The direction of an equilibrium reaction like the one illustrated in this equation depends upon the relative proportions of reactive materials on the two sides of the equation. Addition of glycogen and phosphoric acid will drive the reaction to the right, whereas addition of glucose-1-phosphate will drive it to the left. If, then, glucose-1-phosphate was withdrawn by another reaction as soon as it was formed, the reaction would tend to run always to the right, forming glucose-1-phosphate. The speed and direction of a process is determined by the relative activity of the various components of the enzyme system as well as the quantities of materials provided. This is the principle by which order is achieved in biological organisms. It is the sum of such a series of reactions that is termed an enzyme system. In such a system provision is also made through these linked reactions to restore to an enzyme the activity which it lost in the course of the single reaction

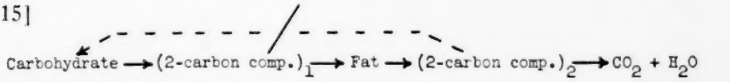
which it implemented. Biological reactions are seldom spontaneously reversible. If, in the course of a series of reactions the original reagents are reconstituted, it is usually by a cyclical or circuitous route. All these points are illustrated in the formation of urea through the agency of arginine (see 9]). It must be recognized that not only the last reaction, but also the first two reactions, ornithine to citrulline and citrulline to arginine, are implemented by enzymes. The reaction begins and ends with ornithine, but not by retracing its steps, which would be futile. In the first two reactions ammonia and CO_2 are accumulated. In the last, through the agency of arginase, they are discharged as urea to be excreted. Furthermore, in this last reaction the molecule of water accumulated in the course of the formation of arginine is used to form the urea, thus leaving the whole system in its original state. In this connection it may be worth while to present a few other examples which will require attention later. Glycogen of the liver is a temporary repository for carbohydrate. Glucose from the blood is built up to glycogen in the liver to be again broken down when required to provide glucose to the blood. The simple equilibrium equation, glucose⇌glycogen, would express the over-all effect of the reaction, but would not be a correct representation of the facts.



Glucose, with the aid of adenosine triphosphate and the enzyme, hexokinase, forms glucose-6-phosphate by an irreversible reaction. From glucose-6-phosphate, glycogen is formed through the intermediate step of glucose-1-phosphate with the aid of the enzymes, phosphoglucomutase, and phosphorylase. These two reactions are apparently reversible. Glucose-6-phosphate can also be changed to fructose-6-phosphate, which may likewise be formed from fructose.* It is evident that through this path fructose can form glycogen. Glucose-6-phosphate can again be converted to glucose by a separate path implemented by the enzyme phosphatase. Glucose-6-phosphate, therefore, occupies a central position through which glucose can pass to glycogen and return to glucose and through which fructose in turn can form either glycogen or glucose. Glycogen of muscle, in contradistinction to glycogen of liver, does not contribute to the glucose of blood. The reason lies in the presence in muscle of another enzyme system for the oxidation of sugar, which begins with the conversion of fructose-6-phosphate to fructose-1 : 6-diphosphate with the aid of adenosinetriphosphate and the enzyme phosphohexokinase. From there the chain of reactions continues through pyruvic acid to carbon dioxide and water. The circuit is, therefore, tapped at the fructose-6-phosphate point by a chain of extremely active reactions. In consequence, the flow through glucose-6-phosphate is entirely diverted from the path, glucose-6-phosphate to glucose, into the channel of oxidation.

Carbohydrate can be converted to fat, but fat does not form important amounts of carbohydrate. Although carbohydrate forms 2-carbon compounds that can be converted to fat,

15]

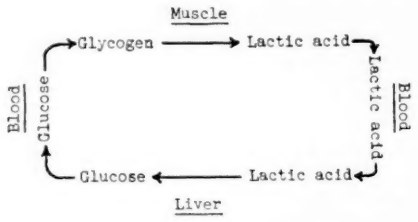


and fat in turn forms 2-carbon compounds that can be burned, the 2-carbon compounds derived from fat cannot be used to form carbohydrate or deriva-

* It has been repeatedly demonstrated that in both normal and diabetic subjects fructose is removed from the blood more rapidly than glucose is, and that in the diabetic subject the blood glucose does not rise so rapidly nor so high after fructose as is does after glucose. Max Miller (personal communication) has recently shown that the liver removes fructose from the blood far more rapidly than it does glucose. At the same time pyruvic acid in the blood increases after fructose, the contribution coming from the liver. This suggests that fructose and glucose are treated differently by the liver; the major part of the former must bypass the common pool of glucose-6-phosphate. This is indicated in equation 14] by the broken line from fructose-6-phosphate to glucose-6-phosphate. This reaction cannot be as freely reversible as has been generally assumed. The chain of reactions proceeding from fructose-6-phosphate to pyruvic acid also must be less strictly confined to muscle than it is here depicted. The tendency for the reaction, glucose-6-phosphate$\rightleftharpoons$fructose-6-phosphate, to proceed predominantly to the right might be referable to inherent characteristics of the enzyme system involved or merely to the fact that fructose-6-phosphate is constantly drawn off for the formation of pyruvate.

tives that can be substituted for carbohydrate. It may be that the 2-carbon compounds formed from carbohydrate are not the same as those formed from fat.

In the absence of oxygen, glycogen of muscle is burned only to lactic acid. The latter is not reconverted to glycogen in the muscle, but is conveyed by the blood stream to the liver where it can be converted to glycogen.



One word about hormones. Although the secretions of the endocrine glands influence chemical reactions, they do not appear to serve as enzymes. They are not the prime movers of metabolism, which does not cease entirely when one or more of the endocrine glands is removed. They appear only to accelerate or retard the actions of the enzyme systems, perhaps by potentiating or inhibiting certain particular enzymes.

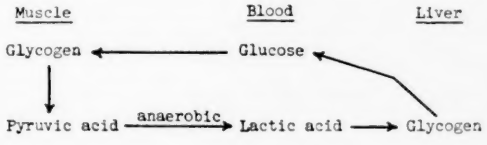
So much for what may be called stage setting. It has been a long and tedious process, but essential to an understanding of the intermediary metabolism and exchange of the various foodstuffs. It is unnecessary to detail all the steps in the estimation of energy metabolism. A few general statements will suffice. Energy is produced by the combustion, that is oxidation, of foodstuffs. The end products of the complete oxidation of carbohydrate and fat, since these substances contain only carbon, hydrogen, and oxygen, are CO_2 and H_2O. The ratio of CO_2 produced to oxygen consumed, the respiratory quotient, as well as the heat produced by consumption of a given amount of oxygen, will vary with the composition of the foodstuff. As far as the two foodstuffs, carbohydrate and fat, are concerned, therefore, the energy produced can be estimated from the oxygen consumed and the CO_2 produced. Protein contains nitrogen and sulfur as well as carbon, hydrogen, and oxygen. In addition to CO_2 and H_2O, therefore, its combustion products will include nitrogen-containing compounds and sulfates which are excreted in the urine, the nitrogen-containing compounds in an incompletely oxidized state. Estimation of the total energy production, then, requires measurement of the oxygen consumed, the carbon dioxide produced and the nitrogen excreted in the urine.

The first steps in the metabolism of carbohydrate have already been described. Not only glucose, but other sugars and some of the products of

the metabolism of protein are converted into glucose or glycogen. The latter is an aggregation of molecules of glucose which is used for the storage of carbohydrate in liver and other tissues. Because of its large molecular weight, it exerts almost no osmotic pressure and, therefore, does not disturb the hydration of cells as glucose itself would. The actual quantity of glycogen in the body is quite small, altogether too small, we now realize, to bear the whole brunt of continuing metabolism. Apart from this, however, there is no other endogenous source of carbohydrate except protein, and the amount ordinarily contributed by protein is quite small. All carbohydrates, as the name implies, are made up of units, each consisting of one atom of carbon and one molecule of water, CH_2O. For example, glucose, $C_6H_{12}O_6$, consists of 6 of these units. It follows that one molecule of oxygen, O_2, is required for the combustion of each unit, forming one molecule of carbon dioxide, CO_2, and one of water, H_2O. Since molecular equivalents of ideal gases occupy equal volumes, the respiratory quotient of carbohydrate is 1.000. The caloric value of 1 gram of carbohydrate equals about 4.1 Calories.

By reference to 14] it will be seen that when glycogen is burned in the muscles it does not pass through glucose as an intermediary step, but through glucose phosphates and fructose phosphates to pyruvic acid. Numerous intermediate steps between fructose-1:6-phosphate and pyruvic acid have been omitted. From this point several alternative paths present themselves. If the supply of oxygen is inadequate, complete oxidation of the pyruvic acid is impossible. Instead it is converted to lactic acid.

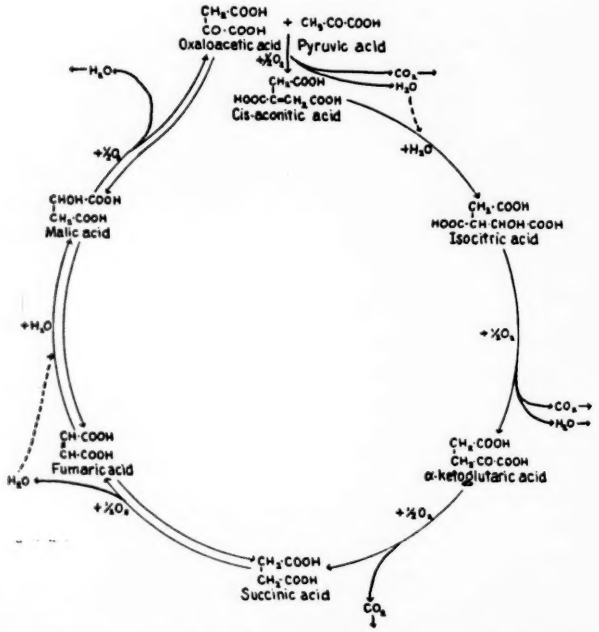
17]



This releases part of the energy of the carbohydrate, but leads to a dead end because the lactic acid cannot be further burned nor can it be reconverted to glycogen in the muscle as Hill and Meyerhoff once conceived. Instead it must be conveyed to the liver for such reconversion. This is apparently the only means of supplying any reasonable amount of energy in the absence of oxygen. In itself it would be a sufficient reason for the provision of a store of carbohydrate in the muscles. Anaerobic exercise is not unusual. It is required for any quick spurt of activity. For instance, a 100-yard sprint is almost entirely anaerobic exercise.

Sustained exercise with carbohydrate as fuel requires oxygen and from pyruvate onwards follows an altogether different channel, the Krebs' cycle, so named from its discoverer.

18]



It has been drawn in circular form to emphasize its cyclical character and to bring out its machine-like nature. It is unnecessary to dwell on all the compounds in this circle, but only some of its characteristics. It is pictured as proceeding clockwise from the top, the 12 o'clock position. At this point oxaloacetic acid, a 4-carbon group, comes up on the wheel to combine with pyruvic acid. Before proceeding further, however, these two acids deserve some attention. In the course of its metabolism, glucose, originally a 6-carbon compound, has been broken in half, leaving pyruvic acid, a 3-carbon compound.

It will be noted first that if a molecule of CO_2 is added to pyruvic acid, it can form oxaloacetic acid.

Pyruvic acid *Oxaloacetic acid*

$$CH_3\text{-}CO\text{-}COOH + CO_2 \longrightarrow \begin{matrix} CH_2\text{-}COOH \\ | \\ CO\text{-}COOH \end{matrix}$$

19] $3C+4H+3O + 1C+2O = 4C+4H+5O$

Pyruvic acid

$$CH_3\text{-}CO\text{-}COOH + 5O \longrightarrow 3CO_2+2H_2O$$

$$3C+4H+3O + 5O = 3C+4H+8O$$

Since oxaloacetic acid is the end and beginning of this cycle, it follows that the cycle can be primed or renewed at any time by means of a combination of pyruvic acid and CO_2. To oxidize pyruvic acid completely will require five atoms of oxygen, five O, producing three molecules of CO_2, and two of water. Let us assume, then, that oxaloacetic acid to start this cycle has been provided by combination of pyruvic acid with CO_2 and that another pyruvic acid has been fed to the oxaloacetic acid on the wheel and that this has started to go around the cycle. At the very beginning, as you will see, it takes on one O and loses a CO_2 and an H_2O as the oxaloacetate and pyruvate combine to form the next component, cis-aconitic acid. Forget for the moment these compounds and consider only the oxygens that enter and the CO_2's that come off. Exactly five oxygen atoms are taken up: one at the very beginning; another at about 3 o'clock, after isocitric acid; a third at 5 o'clock, after α-ketoglutaric; a fourth at 7 o'clock after succinic; and the fifth at 10 o'clock after malic acid. In the same manner three CO_2's are given off: one at the beginning, one at 3 o'clock, and one at 5 o'clock. It can be shown in the same way that two molecules of water are lost. In other words, the pyruvic acid fed to the oxaloacetic acid has been oxidized precisely and completely, leaving the original component, oxaloacetic acid, intact and ready to start another cycle to burn another molecule of pyruvic acid. The cycle can, therefore, be regarded as a machine for the combustion of pyruvic acid, a combustion device. Undoubtedly, the energy required to run this device is derived from the combustion of the pyruvic acid. But all of the energy from this reaction is not dissipated in this futile manner. This is not the actual machinery of muscular activity, but the power plant. The contraction of muscle itself, towards which this energy is directed, probably resides in some less well understood reactions in which the proteins of the muscle are involved. A small amount of pyruvic acid will be required from time to time to replace oxaloacetic acid which may have inadvertently been consumed on the way; otherwise the machinery is self-perpetuating.

Although pyruvic acid, the product of carbohydrate, would be required to form oxaloacetic acid, other fuel might equally well be burned in the Krebs' combustion device. This indeed seems to be the case. Suppose—and this is a somewhat hypothetical reaction—the two-carbon acetic acid were added to oxaloacetic acid.

Acetic acid + *Oxaloacetic acid* + H − H_2O ⟶ *Cis-aconitic acid*

CH_3-COOH + CH_2-COOH (CO-COOH) + H − H_2O ⟶ CH_2-COOH (HOOC-C=CH_2COOH)

20] $2C+4H+2O$ + $4C+4H+5O$ + H − 2H − O = $6C+7H+6O$

Pyruvic acid ⟶ *Acetic acid*

CH_3-CO-COOH + O − CO_2 ⟶ CH_3-COOH

$3C+4H+3O$ + O − C − 2O = $2C+4H+2O$

By the addition of an atom of H and loss of a molecule of water, it could form the next acid in the cycle, cis-aconitic, and continue the round. At the end the acetic acid would have been burned, leaving oxaloacetic acid again. Although this particular reaction is hypothetical, it is now established that 2-carbon compounds are produced from carbohydrate and are oxidized, presumably over the Krebs' cycle. To carry the hypothetical reaction further, we may picture pyruvic acid as being converted to acetic acid by the addition of one atom of oxygen and the loss of a molecule of CO_2. Acetic acid might be quite suitable as fuel for this combustion device, but quite incapable of replacing oxaloacetic acid, that is keeping up the machinery. Certain work of Hastings with isotopes suggests that this is indeed the case. To replace oxaloacetate, pyruvate is required. I would emphasize again that, although these last reactions serve as an illustration of the facts, they cannot be accepted as a precise representation like that of the Krebs' cycle. From this point on the argument will have to be conducted in such approximate or symbolic terms.

Attention must now be diverted to the metabolism of fat. The structure of fat and fatty acids has already been shown. If the structure of a fat is examined, it is at once apparent that it contains very little oxygen in proportion to carbon and hydrogen. For example, tristearin, a fat composed of the saturated 18-carbon stearic acid, has the formula $C_{57}H_{110}O_6$. To oxidize this completely to CO_2 and H_2O will require the addition of 81.5 molecules of O_2, which yield 57 molecules of CO_2. The respiratory quotient will, therefore be $\frac{57.0}{81.5} = 0.70$. The respiratory quotients of various fats lie between 0.70 and 0.71. Combustion of one gram of fat yields about 9.3 Calories.

If the structures of carbohydrate and fat are compared, it is immediately evident that conversion of carbohydrate to fat must involve loss of oxygen. By comparison of the caloric values of the two foodstuffs it is also apparent that energy must be expended in this conversion.

To provide the carbons of tristearin, $C_{57}H_{110}O_6$, from glucose, $C_6H_{12}O_6$, would require 9.5 molecules of glucose. The total molecular weight of tristearin is 890, that of glucose is 180. In terms of gram molecules, therefore, $9.5 \times 180 = 1710$ gm. of glucose are needed to form 890 gm. of tristearin. The caloric value of 890 gm. of tristearin $= 890 \times 9.3 = 8270$ Calories, while 1710 gm. of glucose yields only $1710 \times 3.8 = 6498$ Calories.* This would leave a deficit of $8270 - 6498 = 1772$ Calories. This means that 1772 extra Calories must be expended in the process of conversion. If this is to be provided by glucose, it will require the combustion of $1772/3.8 = 466.6$ gm. of glucose, equivalent to $\frac{466.6}{180} = 2.6$ mole-

* The caloric value of 1 gm. of glucose is 3.8 Calories. The factor generally used for carbohydrate, 4.1, applies strictly to starch.

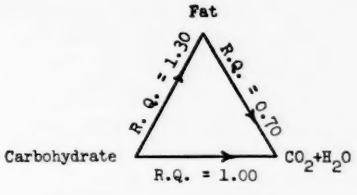
cules. In other words, in addition to the 9.5 molecules of glucose required to provide the structure of the fat, another 2.6 molecules, or almost 30 per cent more, must be burned to effect the conversion. These actual figures are of less importance than the fact that fat cannot be formed from carbohydrate unless some carbohydrate is burned. Because oxygen is given off in this conversion, the respiratory quotient is greater than 1.

As a means of storing fuel, fat has peculiar advantages. A given weight of fat contains $9.3/4.1 = 2.3$ times as many calories as does an equal weight of carbohydrate. The latter, furthermore, must be held in solution. For this purpose about 3 gm. of water are required for each gram of glycogen. Fat, on the other hand, is insoluble, requiring no water. In terms of tissue, therefore, nine Calories of fat occupy the same space or weight as one Calorie of carbohydrate. This constitutes the major problem in the treatment of obesity. In the food, fat conceals itself so effectually; from the body it can be removed so slowly. A single pound of fat represents 4870 Calories. To eliminate this pound in one week an individual must have a dietary deficit of about 700 Calories per day. To lose 50 pounds at this rate will take almost a year.

It was long believed that formation of fat from carbohydrate was a relatively rare occurrence, a process that was reserved altogether for the storage of surplus carbohydrate. It is now recognized that it is continuously active. In fact, it is probable that in the course of a day the greater proportion of carbohydrate consumed is converted to fat. Attention has been called to the fact that the amount of glycogen, the only endogenous reserve of carbohydrate, in the body is small. Under ordinary circumstances, moreover, it remains relatively constant. Quite obviously, then, the carbohydrate eaten at meals cannot be stored entirely as glycogen and expended between meals as carbohydrate. The only form in which it can be stored is fat. This fact was missed because respiratory quotients greater than 1.0 were rarely encountered. The point was overlooked that if fat were being burned and formed at the same time, the respiratory quotient would not have to be 1.0, but would be a resultant of the over-all metabolism. The end result of a chemical reaction, both in energy production and in other respects, is the same, regardless of the intermediary reactions that may occur.

The total R.Q., oxygen consumption, CO_2 production and energy expenditure will be the same whether a given amount of carbohydrate is burned directly or is first converted to fat and then burned. This is symbolized thus:

21]



When a fatty acid is burned, it appears first to be broken down into 2-carbon compounds, not stepwise, two carbons at a time, but in an explosive fashion, just as glycogen is broken down to glucose phosphate.

22] **Stearic acid, $C_{18}H_{36}O_2$** ⟶ 9(2-carbon compounds)

Route 1 (Ketosis)

2(2-carbon compounds) = 1(4-carbon compound) ⟶ Burned to $CO_2 + H_2O$
acetoacetic acid
β-hydroxybutyric acid

Liver — Tissues

Route 2 (Direct combustion)

2-carbon compounds ⟶ Burned to $CO_2 + H_2O$

Tissues

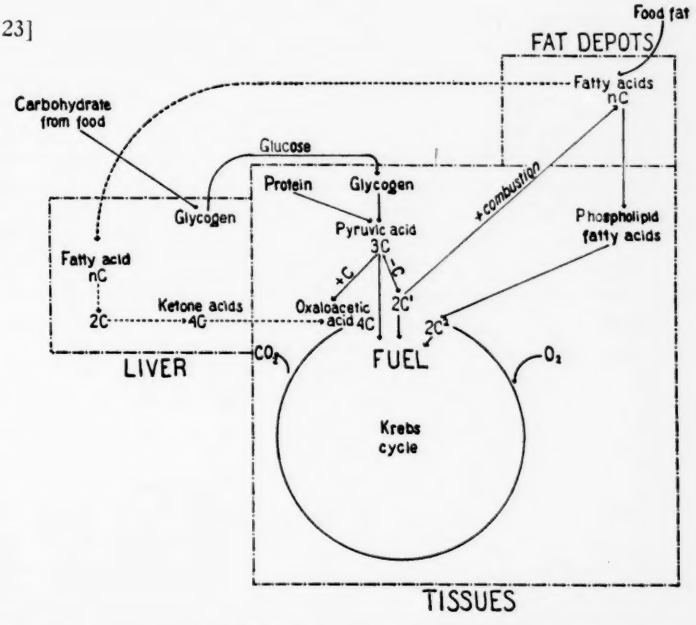
For example, stearic acid with 18 carbons would break down into nine 2-carbon fragments. In the liver these 2-carbon groups may again be coupled to form the 4-carbon compounds, acetoacetic acid and β-hydroxybutyric acid, which are conveyed to the tissues where they are oxidized. This process is exaggerated in starvation and when no exogenous carbohydrate is taken. It is still further exaggerated when the ability to burn carbohydrate is impaired. It was at one time believed that this constituted the sole route for the combustion of fat. It was demonstrated by Stadie that even in the most severely diabetic subject most of the fat is burned by some more direct process. It has been found that isolated muscle tissue from diabetic animals has a respiratory quotient of 0.70, proving that the muscle is burning fat without the intervention of the liver or ketone production. Presumably, then, fatty acids may be broken down in the tissues into 2-carbon compounds that are burned directly.

The formation of 2-carbon compounds from carbohydrate and their combustion over the Krebs' cycle has already been mentioned. It is such 2-carbon groups from carbohydrate that appear to be used for the formation of fat. It is impossible to cite the evidence for each point in this argument. It should be remarked that they have been established chiefly by labeling various compounds with isotopes of carbon or hydrogen and then determining the fate of these isotopes. If acetic acid tagged with the carbon isotope, C^{14}, is given to an animal, the C^{14} is found in all the fatty acids in the body and in the CO_2 of the expired air as well. This is conclusive proof that acetic acid is used for the formation of fat. The C^{14} also appears in the members of the Krebs' cycle, but no appreciable amounts are found in glycogen. It is, therefore, burned over the Krebs' cycle.

It is now necessary to attempt to integrate the functions of the three

foodstuffs. In order to achieve such an integration, certain simplifications are necessary. It must be recognized again that these simplifications involve a certain amount of symbolism. The attempted integration is represented diagrammatically thus:

23]



For our purposes the body is divided into three compartments: the general tissues, the liver, and the fat depots. The functions of the liver in the elaboration of protein, production of urea, rearrangement of lipids, etc. are neglected. The only functions considered are those immediately pertinent to processes of energy production, which are performed by the Krebs' cycle in the tissues. This cycle takes up oxygen and gives off CO_2 in the combustion of appropriate fuel that is fed to it. This fuel is pictured as entering the top of the cycle. In order that the fuel may be burned, however, the machinery of the cycle must be intact, which means that the 4-carbon compound, oxaloacetic acid, must be replenished. Glycogen of the liver is formed from the food and conveyed as glucose to the tissues, where it is again converted to glycogen. This is broken down to the 3-carbon compound, pyruvic acid, which has the capacity both to replenish oxaloacetic acid and to serve as fuel. With an adequate amount of pyruvic acid, therefore, the whole system will run without further assistance. The supply of pyruvic acid is supplemented continuously by protein, the quan-

tity derived from this source depending on the quantity of protein catabolized. The pyruvic acid which is not required to replenish oxaloacetic acid or to supply fuel directly and immediately is represented as forming a 2-carbon group, 2-C^1, which is used for the formation of fatty acids which, together with fat derived from food, are deposited in the fat depots. This formation of fat, however, requires energy which can only be secured by the combustion of a certain proportion of the 2-C^1 in the Krebs' machinery. 2-C^1 can be burned in this machinery, but cannot be used to replenish oxaloacetic acid. From the fat depots fatty acids are conveyed to the phospholipids of the tissues. When they are needed for combustion, these are also broken down to 2-carbon compounds, 2-C^2, which, like 2-C^1, can be burned, but cannot form oxaloacetic acid. There is an alternate, less used path, indicated by broken lines, by which fatty acids are conveyed to the liver. Here they are broken into 2-carbon groups which are immediately coupled to 4-carbon groups, the keto acids, acetoacetic and β-hydroxybutyric, which can be used to replace oxaloacetic acid, thereby enabling the 2-carbon groups from the phospholipids to be burned in the tissues. For this purpose, however, the keto acids cannot be utilized as efficiently as pyruvic acid can. Let me reiterate that the interplay of the foodstuffs cannot yet be defined with such precision as the diagram suggests, but that the principles suggested by the diagram are consistent with known chemical reactions and physiological and pathological phenomena.

If an individual is given large amounts of carbohydrate or carbohydrate and fat, but no protein, the metabolism of protein is, as was remarked before, reduced to a minimum. None is wasted for the formation of pyruvic acid and energy production. If the individual is given no food or no carbohydrate to provide pyruvic acid, the catabolism of protein immediately increases. At the same time fat is mobilized to the liver over the broken line and the formation of ketones increases. This suggests that these substances are being called upon to provide the materials to maintain the Krebs' cycle, which have been represented in this diagram as pyruvic acid and oxaloacetic acid. That they are not being provided for fuel purposes can be easily demonstrated. To reduce the consumption of protein and the formation of ketone acids, so long as the subject has adequate stores of fat in his body, it is not necessary to give enough carbohydrate to meet his fuel requirements, but only a small fraction of this quantity. As little as 100 grams of carbohydrate daily will prevent the previously well-nourished person from wasting excessive amounts of protein and from ketosis. The remainder of his caloric needs will be provided by fat from his adipose tissues. Evidently this carbohydrate is needed for operative purposes, represented here by the formation of pyruvic and oxaloacetic acids.

At this point it becomes apparent that protein is spent with more economy than the other foodstuffs are. In fact, ketosis, the provision from fat of substitutes for operative necessities usually derived from carbo-

hydrate, may be regarded as an expedient to reduce the destruction of protein.

If the pancreas is removed, the ability to burn carbohydrate is reduced to a minimum. Nevertheless, the machinery of the Krebs' cycle continues. Isolated muscle from the depancreatized animal retains the ability to contract, but its respiratory quotient approaches 0.70, indicating that the source of energy is almost entirely fat. Nevertheless, in this muscle all the elements of the Krebs' cycle can be identified and, if proper elements are added, they are utilized. The operating metabolism, therefore, continues, though possibly less efficiently than usual, but the fuel is changed. At just what point or points the continuity of the chains of reactions is broken has not been established with certainty, but the consequences are quite clear. One of these consequences particularly deserves attention because its significance has been too little appreciated. With loss of the ability to burn carbohydrate, formation of fat from carbohydrate is abolished. The implications of this disorder are profoundly important. So long as formation of fat from carbohydrate was regarded as an occasional incident only, little attention was given to it. Controversy instead centered about the question whether fat could form carbohydrate. Those who held the affirmative position in this argument contended that mere interruption of the combustion of carbohydrate could not explain the extreme accumulation of glucose in the blood and the large amounts excreted in the urine by the depancreatized dog and in diabetes mellitus. If, however, formation of fat from carbohydrate is the main pathway for the continuing metabolism of carbohydrate, obstruction of this pathway will immediately cause glucose to back up in the blood and to pour into the urine. Direct evidence that fat forms appreciable quantities of carbohydrate has not been adduced. Studies with isotopic labeled compounds have lent strength to those who have held that conversion of carbohydrate to fat is essentially an irreversible reaction.

In the diabetic the formation of pyruvic acid also is impaired. The source of oxaloacetic acid for the maintenance of the operative machinery of the Krebs' cycle is therefore cut off. In a futile effort to break through this obstruction, the destruction of protein increases to augment the deficient supply of pyruvic acid. Effective relief is afforded by fat, which is mobilized in large quantities from the depots and shipped over the broken-lined path to the liver, where it is broken down to provide ketone acids which can be used to form oxaloacetic acid, thereby enabling the Krebs' cycle to continue and to burn the 2-carbon compounds derived from fat. Under these circumstances, fat, virtually unsupported, sustains energy-production and the operative functions usually carried by carbohydrate.

This substitution, although it enables the subject to survive and subsist for a time, has serious disadvantageous features. In the first place, while the production of ketone bodies may mitigate the destruction of protein, it does not eliminate it. The combination of excessive nitrogenous waste

products and glycosuria provokes a dehydrating diuresis. Secondly, whether because ketone acids cannot be utilized as efficiently as pyruvic acid, or merely because of the overgenerous spirit of the liver, these acids are poured into the blood by this organ so much more rapidly than they are burned by the tissues that they accumulate in the blood, producing acidosis.

The novel position of importance achieved by fat in the continuing metabolism is an intriguing subject for speculation. The foremost point for consideration is that, except for the essential fatty acids, which have a purely operative rôle, fat is not altogether a separate foodstuff; it is equally an intermediary compound in the metabolism of carbohydrate. It is rather banal to state that this is one more good reason why more attention should be given to the metabolism of the lipids, both in physiology and the clinic. It is at least fair to suggest that the metabolism of the two foodstuffs should no longer be categorically separated. The glucose-fed animal, so dear to physiologists and biochemists, is not just what it used to be. Concepts of the rôle of glycogen require modification. It has already fallen far from the high estate it enjoyed in the reign of Hill and Meyerhoff, when it was the sole fuel of muscle. Is it now to be displaced from its position as the chief source of continuing energy? This possibility must at least be given open-minded consideration. An adult male, under ordinary conditions, does not develop ketosis of any important degree until he has starved 48 hours or more, although the glycogen in his body could supply his energy requirements for only a fraction of this time. After an overnight fast three-fifths of the energy production is sustained by fat. If it were not, the glycogen stores would be entirely exhausted.

When there is not a continuous supply of exogenous carbohydrate, the endogenous supply is used with increasing economy as it becomes depleted. What little remains is reserved for essential operative purposes. In this respect the capacity for economy varies from species to species, being most highly developed in carnivores who habitually subsist on diets almost devoid of carbohydrate, compelling them to derive what carbohydrate they need for operating metabolism from protein. Within species similar variations exist. Women and children are more susceptible than men to ketosis, presumably because they are more prodigal with carbohydrate. Pregnancy exaggerates this characteristic of women.

Although muscular exercise is not sustained by carbohydrate alone, it does appear to create a preferential demand for carbohydrate, so long as this is freely available. When Courtice and Douglas walked 10 miles before breakfast, their respiratory quotients rose quite rapidly, to drop as soon as they rested to figures that indicated that 90 per cent of their resting fuel requirements were being supplied by fat. When they resumed walking, the respiratory quotients rose slightly again. If they took carbohydrate while they were walking, this was rapidly expended. This exercise of Courtice and Douglas was not severe enough to induce any appreciable production

of lactic acid. Carbohydrate is not only utilized preferentially for muscular exercise, its utilization is facilitated by exercise. The diabetic patient requires less insulin when he is physically active. This is one of the chief arguments against the common practice of admitting patients to hospitals for regulation of diabetes.

Certain hormones accelerate the expenditure of carbohydrate. Among these epinephrine has a unique action. It appears to inhibit the oxidative metabolism while augmenting the anaerobic breakdown of glycogen. It therefore increases the production of lactic acid and provokes ketosis. Thyroxine seems to behave somewhat like exercise, increasing the total energy-production and promoting the preferential expenditure of carbohydrate. Insulin is, however, the most specific and potent stimulus to the combustion of carbohydrate. Whereas the action of muscular exercise diminishes as the glycogen stores become depleted, the action of insulin is not similarly limited. Moreover, insulin retards the breakdown of protein and therefore impedes the restoration of glycogen from non-carbohydrate sources. It seems also to facilitate the formation of fat from carbohydrate, thereby further dissipating carbohydrate stores. In the absence of an extraneous supply of carbohydrate, therefore, insulin is the most powerful known deglycogenating force.

The reduction of carbohydrate oxidation to a minimum (e.g. in starvation), if it does not involve a complete transformation of the metabolic processes, at least requires a complete revision of their organization or adjustment. The flow through pyruvic acid to oxaloacetate and from 2-C^1 to fat must be greatly retarded, while the channels from fat through 2-C^2 and to ketones in the liver are opened up. The relative speeds of the various enzyme systems have all been changed to meet new conditions. So profound are these changes that they cannot be immediately reversed. If a dose of glucose is given to an adult male who has been starved for 48 hours, the blood sugar curve will resemble that of a diabetic and some sugar is likely to appear in the urine. Some time must elapse before normal utilization of carbohydrate is resumed. A similar state of starvation diabetes can be induced more easily in women or children. Appearance of this state can be accelerated by exercise. In the experiments of Courtice and Douglas cited above, glucose tolerance was distinctly reduced after the morning walks. The glycosuria not uncommonly shown by patients with hyperthyroidism is probably a manifestation of starvation. It usually follows breakfast. Although the blood sugar rises excessively after a dose of glucose before breakfast, sugar is subsequently used with great facility. The hyperthyroid patient, owing to accelerated oxidations and preferential expenditure of carbohydrate, depletes his carbohydrate stores more rapidly than the normal person does and therefore exhibits the phenomena of starvation after a simple overnight fast. Perhaps the simplest way to produce starvation diabetes is by the administration of insulin to a starving individual. Of

course, if carbohydrate is given while the insulin is acting, its combustion will be facilitated. But after a hypoglycemic reaction, if the subject is not immediately given carbohydrate, ketosis will regularly appear. At this time a dose of carbohydrate will provoke excessive and prolonged hyperglycemia, a diabetic type of reaction.

At this point it may be profitable to refer briefly to the bearing of this subject on diabetes. In the first place, it should be clear that in order to keep the stream of metabolism flowing in its normal channels, a small, but steady, supply of carbohydrate should be provided, enough to maintain the operative functions of carbohydrate. Long periods of starvation should be avoided, especially if forces are active, which may accelerate the preferential expenditure of carbohydrate or impair its oxidation. In diabetes, oxidation of carbohydrate is regularly impaired; therefore it becomes doubly important to guard against depletion of carbohydrate stores by starvation or forces that accelerate the expenditure of carbohydrate, of which exercise and insulin are the most important.

The subject cannot be dismissed without some mention of the hormones which regulate combustion of carbohydrate. The peculiar effect of epinephrine has already been mentioned. The rôle played by the hormones of the anterior pituitary and adrenal cortex in the regulation of carbohydrate metabolism is an intricate one, but the purification of the active principles of these glands has in recent years enabled considerable progress to be made in the elucidation of their effects.

As is now well known, the anterior pituitary secretes six hormones. Of these, four are trophic hormones; that is to say, they produce their effects by stimulating the secretion of other endocrine organs. The trophic hormones are the two gonadotrophic agents, follicle stimulating hormone and the luteinizing principle, the thyrotrophic hormone and the adrenocorticotrophic hormone (ACTH). Of these trophic hormones only the last two are of any concern in the regulation of metabolism. In addition to these trophic agents, the anterior pituitary also secretes two other hormones that act directly on tissues that are receptive to their action. These are the lactogenic hormone and the so-called growth hormone, which in reality is a metabolic hormone with a profound effect on protein synthesis. Since the lactogenic hormone probably only plays a significant rôle in the mammal in the special processes related to milk secretion, the only hormones of the anterior pituitary that require consideration as metabolic hormones are the thyrotrophic, the adrenocorticotrophic, and the growth hormone.

Since the thyrotrophic and adrenocorticotrophic hormones merely increase the secretory rate of the thyroid and adrenal cortex respectively, their effects on metabolism are identical with those produced by thyroxine or the adrenal cortical steroids. The effects of the thyroid hormone have been described above.

The adrenal cortical hormones of the type of cortisone, which possess an oxygen atom in the third ring of the steroid nucleus, have the property of accelerating in fasting animals the rate of formation of glucose from the tissue proteins. In fed animals they also inhibit the rate of utilization of carbohydrate. When either ACTH or cortisone is given in excess to normal animals or humans, a temporary diabetic state may be produced. This is also characterized by an extreme degree of resistance to insulin. In partially depancreatized animals, or in individuals with a potential or mild diabetes, these effects on carbohydrate metabolism are much more severe.

The growth hormone, when administered to normal cats or dogs in quite small amounts, will produce in the course of a few days either a very severe temporary diabetes or a permanent one. When the latter occurs, it can be demonstrated that this is a consequence of the widespread degeneration of the islets of Langerhans. It is believed that in susceptible species such degeneration is due to the marked hyperglycemia that is associated with the administration of growth hormone to fed animals.

The effects of growth hormone on carbohydrate metabolism are of a kind that suggest that this hormone inhibits the utilization of glucose by the skeletal muscles. Thus it has been found that the glucose utilization of isolated skeletal muscle is markedly reduced in animals previously treated with this hormone, while its injection is followed by a depression of the respiratory quotient.

It has been suggested by the Coris that both the adrenal cortical steroids and a pituitary hormone (growth?) inhibit the enzyme hexokinase. This enzyme is of central importance in carbohydrate metabolism since it catalyzes the formation of glucose-6-phosphate from glucose, a step that is essential for normal utilization of glucose (see 14]). Although this is a most intriguing suggestion, no definite evidence has been adduced to support it.

It is reasonably certain, however, that it is not the hormones themselves that act as enzymes.

References

It would be clumsy to implement a philosophical article of this kind with a conventional bibliography. In a sense it is a natural consequence of a concept first proposed by the author in an article entitled *A new frame for metabolism*, in 1941. Much of the material is included in *Quantitative clinical chemistry, interpretations*, Vol I, Ed. 2. The chief subsequent references are listed below.

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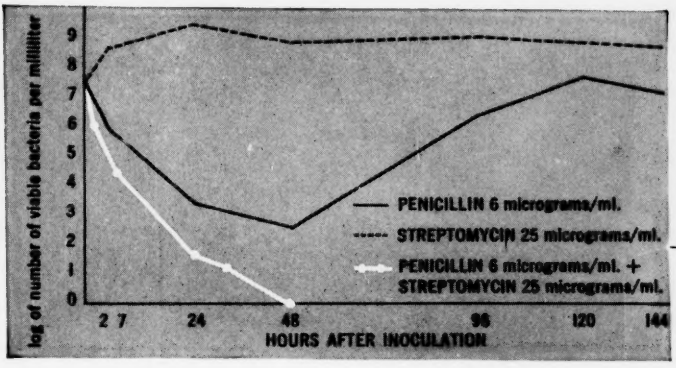

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2. Tompsett, R., and McDermott, W.: Am. J. Med. *7*:371 (Sept.) 1949.

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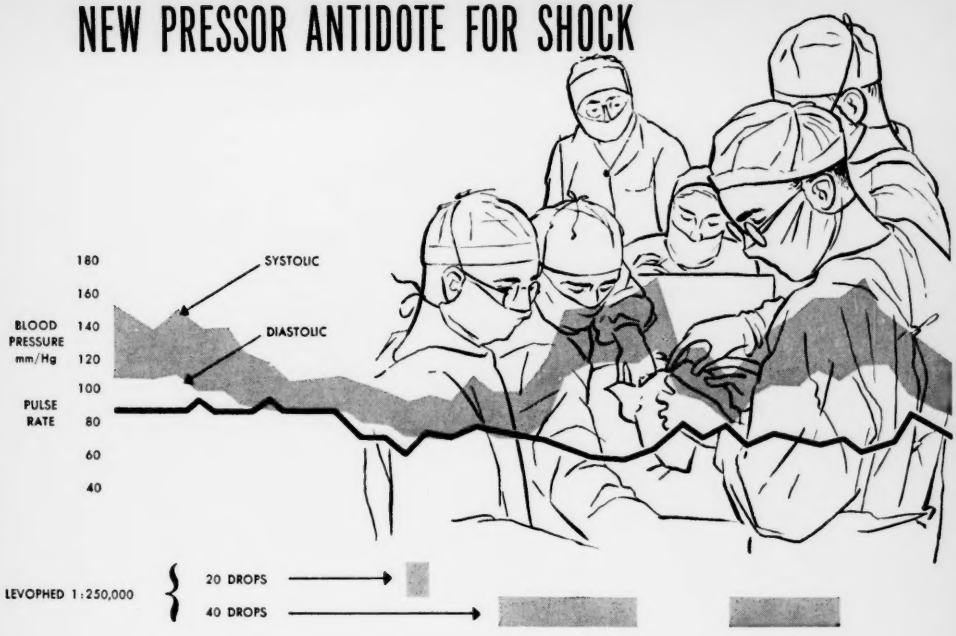

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